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Investigation into the production of bacteriostatic substances by fungi

VI. Examination of the larger Basidiomycetes

BY W. H. WILKINS AND G. C. M. HARRIS

From the Mycology Laboratory, University Department of Botany, Oxford

The present paper, the sixth of the series, gives the results of testing some 700 species of the larger Basidiomycetes for bacteriostatic properties. Initially, the 'juice' extracted from the sporophore was tested and this was followed in certain cases by a test of the metabolism solution produced by the fungus in culture. The results indicate that the larger Basidiomycetes are among the more promising fungus groups which produce antibiotics and that they compare favourably in this respect with the Aspergilli and the Penicillia. Of the 700 species tested approximately 70 are strongly and approximately 100 weakly positive against *Staphylococcus aureus* and/or *Bacterium coli*.

Previous work in the above investigation (Wilkins & Harris, 1942, 1943*b*, 1944*b*) has indicated that the fungal groups which show the most marked antibacterial action are the Penicillia and the Aspergilli, and that the Phycomycetes and the Ascomycetes are relatively inactive. During the seasons 1942–3 work has been done on the larger Basidiomycetes, but whereas the groups previously tested were grown in culture, the Basidiomycetes were first tested as extracts of the sporophores and cultural work was subsequently related to this. In 1942, mycologists in different parts of the country were asked to extract 'juice' from sporophores and about 800 individual fungi were tested. By far the larger number was sent in by our collaborators at Haslemere, Messrs E. W. Swanton and A. A. Pearson, and, as the identification in this case could be depended upon, in 1943 the collection was restricted to ourselves and our Haslemere colleagues only. From Haslemere we received approximately 500 extracts in 1942 and approximately 600 in 1943. In all we have examined 722 species distributed over 96 genera. In all cases the date and place of collection, the name of the person responsible for identification and the date and method of extraction were carefully recorded.

Extraction was usually done by grinding the sporophores in a mortar, either with or without sand, and squeezing about 2 ml. of the juice through fine muslin into a sterile phial. If sent by post one drop of chloroform was added to each phial. At Oxford the extract was tested for its bacteriostatic properties against three representative types of bacteria, *Bacterium coli*, *Staphylococcus aureus* and *Pseudomonas pyocyanea*, by the method already described (Wilkins & Harris, 1943*a*). Briefly the method consists in putting a few drops of the sporophore extract which is to be tested for its bacteriostatic properties into a circular hole 11 mm. diam. cut in the centre of a plate of agar incorporated with the bacterium against which the test is to be made and incubating at 37° C. overnight. During this period bacteriostatic substances, if any, will diffuse outwards from the hole and the bacteria will grow into colonies making the agar opaque. The bacterial growth will be inhibited in the region of diffusion and so there will be, round the hole, a circular, clear, bacteria-free zone, the width of which will indicate the relative concentration of the substance. It had been found in 1942 that individual sporophores of the same species did not always give the same result, hence the decision to repeat the experiment in 1943. It was inevitable that certain species should be found in 1942 only and certain others in 1943 only, but even so approximately 400 species were duplicated in the 2 years. In some cases several sporophores of the same species were tested while in others only one sporophore was available, but, on the whole, the repetition confirmed the original test. Those species which are particularly promising are being examined for chemical and therapeutic properties at the Oxford School of Pathology.

It is not suggested that extracts of any given fungus sporophores could ever be an adequate or dependable source of bacteriostatic substances of therapeutic significance, but it is possible that the information obtained may lead to subsequent chemical synthesis of such substances or that the fungus might be cultured in the laboratory and from the metabolism solution a purified substance of therapeutic importance might be isolated (cf. Penicillin). In certain instances it was desirable to have relatively larger quantities of a given species. Fresh sporophores sent by post had invariably lost their bacteriostatic properties, but if they were dried before sending, the dried material when ground up with water gave, in most cases, a result comparable with that obtained from fresh material tested immediately after collection.

The results of the species tested during the 2 years are given in Table 1. In each year the test was made against each of the three bacteria already mentioned, but as only five species, *Amanita solitaria*,

TABLE 1. *List of fungi tested*

	Bact. coli	*Staph. aureus*	*Bact. coli*	*Staph. aureus*
Acia stenodon (Pers.) Bourd. & Galz.	–	–	o	×
A. uda (Fr.) Bourd. & Galz.	–	–	o	o
Amanita echinocephala Vitt.	–	–	×	×
A. mappa (Batsch.) Fr.	o	o	o	o
A. muscaria (Linn.) Fr.	o	o	o	o
A. pantherina (D.C.) Fr.	o	o	o	o
A. pantherina forma *robusta* n.f.	–	–	o	×
A. phalloides (Vaill.) Fr.	o	o	o	o
A. porphyria (A. & S.) Fr.	o	o	o	o
A. rubescens (Pers.) Fr.	o	o	o	o
A. solitaria (Bull.) Fr.	–	–	o	×
A. spissa Fr.	o	o	o	o
A. virosa Fr.	×	×	–	–
Amanitopsis fulva (Schaeff.) W. G. Sm.	o	o	o	o
A. strangulata (Fr.) Roze.	o	o	–	–
A. vaginata (Bull.) Roze.	o	o	o	o
Androsaceus androsaceus (Linn.) Pat.	o	o	o	o
A. rotula (Scop.) Pat.	o	o	–	–
Anellaria separata (Linn.) Karst.	o	o	o	o
Armillaria mellea (Vahl.) Fr.	o	o	o	o
A. mucida (Schrad.) Fr.	o	o	o	o
A. ramentacea (Bull.) Fr.	o	o	–	–
A. robusta (A. & S.) Fr.	o	o	–	–
Auricularia auricula-Judae (Linn.) Schroet.	o	×	o	o
A. mesenterica (Dicks.) Fr.	–	–	o	o
Bolbitius vitellinus (Pers.) Fr.	o	×	o	×
Boletus albidus (Roques.) Quél.	×	×	×	×
B. appendiculatus (Schaeff.) Fr.	–	–	o	o
B. aurantiacus (Bull.) Roques. (=*versipellis* Fr. pro parte)	o	o	o	o
B. badius Fr.	o	o	o	o
B. bovinus (Linn.) Fr.	o	o	o	o
B. calopus Fr.	–	–	×	×
B. chrysenteron (Bull.) Fr.	o	o	o	o
B. cramesinus Secr. (=*gentilis* Quél.)	–	–	o	o
B. duriusculus Schulz.	o	o	–	–
B. edulis (Bull.) Fr.	o	×	o	o
B. edulis var. *bulbosus* (Bull.) Big. & Guill.	–	–	o	o
B. elegans (Schum.) Fr.	o	o	o	o
B. erythropus (Pers.) Quél.	o	o	o	o
B. granulatus (Linn.) Fr.	o	o	o	o
B. impolitus Fr.	o	o	o	o
B. luridus (Schaeff.) Fr.	o	o	o	o
B. luteus (Linn.) Fr.	o	o	o	o
B. nigrescens Roze & Rich.	o	o	o	o
B. piperatus (Bull.) Fr.	o	o	o	o
B. pruinatus Fr.	o	o	o	o
B. pseudo-scaber Kallenbach (=*scaber* (Bull.) Quél.)	o	o	–	–
B. pulverulentus Opatowski	o	o	o	o
B. purpureus Fr.	–	–	o	o
B. reticulatus (Schaeff.) Boud.	o	o	o	o
B. rubinus W. G. Sm.	–	–	o	o
B. rufescens (Secr.) Konrad. (=*versipellis* Fr. pro parte)	o	o	–	–
B. satanus Lenz.	×	×	×	×
B. scaber (? Bull.) Fr. non Quél.	o	o	o	o
B. spadiceus (Schaeff.) Fr.	o	o	–	–
B. subtomentosus (Linn.) Fr.	o	o	o	o
B. subtomentosus var. *radicans* (Krombh.) Massee	–	–	o	o
B. variegatus (Swartz.) Fr.	o	o	o	o
B. versicolor Rostk.	o	o	o	o
B. viscidus (Linn.) Fr.	o	o	o	o
Bovista nigrescens Pers.	o	o	–	–
B. plumbea Fr.	–	–	o	o
Calocera cornea (Batsch.) Fr.	–	–	o	o
C. viscosa (Pers.) Fr.	o	o	o	o
Cantharellus cibarius Fr.	o	×	o	×
C. cibarius var. *albus* Fr.	–	–	o	o
C. cinereus (Pers.) Fr.	o	o	o	o
C. tubaeformis Fr.	o	×	o	×
C. umbonatus (Gmel.) Fr.	o	o	–	–
Claudopus variabilis (Pers.) W. G. Sm.	o	o	o	o
Clavaria argillacea (Pers.) Fr.	o	o	o	o
C. botrytis (Pers.) Fr.	o	o	o	o
C. cinerea (Bull.) Fr.	o	o	o	×
C. corniculata (Schaeff.) Fr.	o	o	o	o
C. corniculata var. *pratensis* (Fr.) C. & W.	o	o	o	o
C. cristata (Holmsk.) Fr.	o	o	–	–
C. flaccida Fr.	o	o	–	–
C. formosa (Pers.) Fr.	o	o	–	–
C. fragilis (Holmsk.) Fr.	o	o	–	–
C. fumosa (Pers.) Fr.	–	–	o	o
C. fusiformis (Sow.) Fr.	o	o	–	–
C. inaequalis (Müller) Fr. sec. Cotton	o	o	o	o
C. Kunzei Fr.	o	×	–	–
C. persimilis Cotton	–	–	o	×
C. pistillaris (Linn.) Fr.	o	o	o	o
C. rugosa (Bull.) Fr.	o	o	o	o
C. stricta (Pers.) Fr.	o	o	o	o
C. vermicularis Fr.	–	–	o	o
C. versatilis Quél.	o	o	o	o
Clitocybe aggregata (Schaeff.) Fr.	o	o	–	–
C. aurantiaca (Wulf.) Studer	×	o	×	o
C. brumalis Fr.	o	×	o	o
C. candicans (Pers.) Fr.	×	×	o	×
C. cartilaginea (Bull. non Fr.) Bres.	o	o	–	–
C. cerussata Fr.	–	–	o	o
C. clavipes (Pers.) Fr.	×	×	o	×
C. connata (Schum.) Fr.	o	×	–	–
C. cyathiformis (Bull.) Fr.	o	×	o	×
C. dealbata (Sow.) Fr.	o	o	o	o

TABLE 1 (*cont.*)

	Bact. coli	*Staph. aureus*	*Bact. coli*	*Staph. aureus*
Clitocybe diatreta Fr.	o	o	o	o
C. ditopus Fr.	×	o	×	×
C. flaccida (Sow.) Fr.	—	—	×	×
C. flaccida var. *lobata* (Sow.) Cke.	—	—	×	×
C. fragrans (Sow.) Fr.	o	o	o	o
C. gallinacea (Scop.) Fr.	o	×	—	—
C. gangraenosa Fr.	o	×	—	—
C. geotropa (Bull.) Fr.	o	o	o	o
C. gilva Fr.	o	o	—	—
C. hirneola Fr.	—	—	o	o
C. infundibuliformis (Schaeff.) Fr.	o	×	o	×
C. inornata (Sow.) Fr.	o	×	—	—
C. inversa (Scop.) Fr.	×	×	×	×
C. maxima (Fl. Wett.) Fr.	o	o	o	o
C. metachroa (Fr.) Berk.	o	o	o	×
C. nebularis (Batsch.) Fr.	o	o	o	o
C. obsoleta (Batsch.) Fr.	×	×	o	×
C. odora (Bull.) Fr.	×	×	×	×
C. phyllophila Fr.	o	o	o	o
C. pithyophila (Secr.) Fr.	o	o	—	—
C. pruinosa (Lasch.) Fr.	×	×	—	—
C. vermicularis Fr.	o	o	—	—
C. vibecina Fr.	×	×	×	×
Clitopilus prunulus (Scop.) Fr.	o	×	o	o
Collybia acervata Fr.	o	o	o	o
C. ambusta Fr.	o	o	o	o
C. butyracea (Bull.) Fr.	o	o	o	o
C. distorta Fr.	o	o	—	—
C. extuberans (Batt.) Fr.	o	o	—	—
C. fusipes (Bull.) Fr.	o	o	×	o
C. lancipes Fr.	o	o	—	—
C. maculata (A. & S.) Fr.	o	o	o	o
C. maculata var. *immaculata* Cke.	o	o	o	o
C. misera (Fr.) Lange	o	o	—	—
C. nitellina Fr.	o	o	—	—
C. platyphylla (Pers.) Fr.	×	×	×	×
C. prolixa (Fl. Dan.) Fr.	o	×	o	×
C. pseudo-clusilis Joss. & Konr.	—	—	o	o
C. radicata (Rehl.) Berk.	o	o	o	o
C. rancida Fr.	o	o	o	o
C. tesquorum (Fr.) Bres.	o	o	—	—
C. tuberosa (Bull.) Fr.	o	o	o	o
C. velutipes (Curt.) Fr.	o	o	o	o
Coniophora arida Fr.	—	—	o	o
C. puteana (Schum.) Karst.	o	o	o	o
Coniophorella olivacea (Fr.) Karst.	—	—	o	o
Coprinus atramentarius (Bull.) Fr.	o	o	o	o
C. comatus (Fl. Dan.) Fr.	×	o	o	o
C. macrorhizus (Pers.) Rea	—	—	o	o
C. micaceus (Bull.) Fr.	o	o	o	o
C. narcoticus (Batsch.) Fr.	—	—	o	o
C. niveus (Pers.) Fr.	—	—	o	o
C. picaceus (Bull.) Fr.	o	o	o	o
C. plicatilis (Curt.) Fr.	o	o	o	o
Corticium arachnoideum Berk.	—	—	×	×

	Bact. coli	*Staph. aureus*	*Bact. coli*	*Staph. aureus*
C. atrovirens Fr.	—	—	o	o
C. comedens (Nees.) Fr.	—	—	o	o
C. confluens Fr.	—	—	×	o
C. laeve (Pers.) Quél.	—	—	o	o
C. Sambuci (Pers.) Fr.	—	—	o	o
C. vagum Berk. & Curt.	—	—	o	o
C. (Gloeo.) praetermissum (Karst.) Bres.	—	—	o	o
Cortinarius (Dermo.) anomalus Fr.	o	o	o	×
C. (D.) azureus Fr.	o	o	—	—
C. (D.) caninus Fr.	o	o	—	—
C. (D.) cinnabarinus Fr.	o	o	o	o
C. (D.) cinnamomeus (Linn.) Fr.	o	o	o	o
C. (D.) lepidopus Cke.	—	—	o	o
C. (D.) malicorius (Fr.) Lange	o	o	o	o
C. (D.) ochroleucus (Schaeff.) Fr.	o	o	o	o
C. (D.) phoeniceus (Bull.) Maire	—	—	o	o
C. (D.) sanguineus (Wulf.) Fr.	o	o	o	o
C. (D.) semisanguineus (Brig.) Maire	o	o	o	o
C. (D.) tabularis (Bull.) Fr.	o	o	o	o
C. (D.) uliginosus Berk.	o	o	—	—
C. (Hydro.) acutus (Pers.) Fr.	—	—	o	o
C. (H.) armeniacus (Schaeff.) Fr.	o	×	—	—
C. (H.) castaneus (Bull.) Fr.	o	o	o	o
C. (H.) damascenus Fr.	o	o	o	o
C. (H.) decipiens (Pers.) Fr.	o	o	o	o
C. (H.) dilutus (Pers.) Fr.	—	—	o	o
C. (H.) duracinus Fr.	o	o	—	—
C. (H.) erythrinus Fr.	o	o	o	o
C. (H.) fasciatus Fr.	o	o	—	—
C. (H.) germanus Fr.	o	o	—	—
C. (H.) holophaeus J. E. Lange	—	—	o	o
C. (H.) jubarinus Fr.	o	o	o	o
C. (H.) Krombholzii Fr.	o	o	—	—
C. (H.) leucopus (Bull.) Fr.	o	o	—	—
C. (H.) obtusus Fr.	o	o	o	o
C. (H.) Reedii Berk.	o	o	—	—
C. (H.) rigens (Pers.) Fr.	o	o	—	—
C. (H.) uraceus Fr.	—	—	o	o
C. (Ino.) alboviolaceus (Pers.) Fr.	o	o	—	—
C. (I.) bolaris (Pers.) Fr.	o	×	—	—
C. (I.) pholideus Fr.	o	o	—	—
C. (I.) suillus Fr.	o	o	—	—
C. (I.) turgidus Fr.	—	—	o	o
C. (Myx.) collinitus (Sow.) Fr.	×	o	×	o
C. (M.) delibutus Fr.	o	o	—	—
C. (M.) elatior Fr.	o	o	o	o
C. (Phleg.) caerulescens Fr.	o	o	o	o
C. (P.) caesiocyaneus Britz.	—	—	o	×
C. (P.) calachrous (Pers.) Fr.	o	o	o	×
C. (P.) cliduchus Fr.	—	—	×	×
C. (P.) croceo-caeruleus (Pers.) Fr.	o	o	—	—

TABLE 1 (*cont.*)

	Bact. coli	*Staph. aureus*	*Bact. coli*	*Staph. aureus*
Cortinarius (*P.*) *crystallinus* Fr.	o	o	o	o
C. (*P.*) *cyanopus* (Secr.) Fr.	o	o	—	—
C. (*P.*) *decoloratus* Fr.	o	o	o	o
C. (*P.*) *elegantior* Fr.	o	o	—	—
C. (*P.*) *emollitus* Fr.	—	—	o	o
C. (*P.*) *fulgens* (A. & S.) Fr.	o	o	o	o
C. (*P.*) *glaucopus* (Schaeff.) Fr.	o	o	o	o
C. (*P.*) *infractus* (Pers.) Fr.	o	o	o	o
C. (*P.*) *intermedius* Rea	—	—	o	o
C. (*P.*) *largus* Fr.	o	o	o	o
C. (*P.*) *multiformis* Fr.	—	—	o	o
C. (*P.*) *orichalceus* (Batsch.) Fr.	o	×	o	o
C. (*P.*) *papulosus* Fr.	o	o	—	—
C. (*P.*) *purpurascens* Fr.	—	—	o	o
C. (*P.*) *rufo-olivaceus* Fr.	—	—	o	×
C. (*P.*) *saginus* Fr.	—	—	o	×
C. (*P.*) *triumphans* Fr.	×	×	×	×
C. (*Tela.*) *armillatus* Fr.	o	o	o	×
C. (*T.*) *biformis* Fr.	—	—	o	o
C. (*T.*) *bovinus* Fr.	—	—	o	×
C. (*T.*) *brunneus* (Pers.) Fr.	o	o	—	—
C. (*T.*) *evernius* Fr.	o	o	o	o
C. (*T.*) *flexipes* Fr.	o	o	—	—
C. (*T.*) *gentilis* Fr.	—	—	o	o
C. (*T.*) *glandicolor* Fr.	o	o	—	—
C. (*T.*) *hemitrichus* Fr.	o	o	o	o
C. (*T.*) *hinnuleus* (Sow.) Fr.	o	o	o	o
C. (*T.*) *iliopodius* Fr.	o	o	—	—
C. (*T.*) *incisus* (Pers.) Fr.	o	o	o	o
C. (*T.*) *malachius* (Fr.) Pearson	o	o	o	o
C. (*T.*) *paleaceus* (Weinm.) Fr.	o	o	o	o
C. (*T.*) *rigidus* (Scop.) Fr.	o	o	o	o
C. (*T.*) *scutulatus* Fr.	o	o	—	—
C. (*T.*) *stemmatus* Fr.	o	o	o	o
C. (*T.*) *torvus* Fr.	o	o	o	o
C. (*T.*) *triformis* Fr.	—	—	o	o
Craterellus cornucopioides (Linn.) Fr.	o	o	o	o
Crepidotus calolepis Fr.	o	o	—	—
C. fragilis Josserand	—	—	×	×
C. mollis (Schaeff.) Fr.	o	o	o	o
Cyathus striatus (Huds.) Pers.	—	—	o	o
Cynophallus caninus (Huds.) Fr.	o	o	o	o
Dacryomyces deliquescens (Bull.) Duby.	—	—	o	o
Daedalea biennis (Bull.) Quél.	—	—	o	o
D. quercina (Linn.) Fr.	o	o	o	o
Eccilia carneogrisea B. & Br.	o	o	—	—
Eichleriella spinulosa (Berk. & Curt.) Burt.	—	—	o	o
Entoloma ameides B. & Br.	—	—	o	o
E. Bloxhamii Berk.	—	—	o	o
E. clypeatum (Linn.) Fr.	o	×	—	—
E. griseocyaneum Fr.	—	—	o	o
E. jubatum Fr. non Cooke	o	o	—	—
E. lividum (Bull.) Fr.	o	o	o	o
E. niderosum Fr.	o	o	o	o
E. niderosum var. *umbilicatum* n.v.	o	o	o	o
E. porphyrophaeum Fr.	o	o	o	o
E. prunuloides Fr.	o	o	—	—
E. rhodopolium Fr.	o	o	o	o
E. sericeum (Bull.) Fr.	o	o	o	o
Exidia glandulosa (Bull.) Fr.	—	—	o	o
E. nucleata (Schwein). Rea	—	—	o	o
Fistulina hepatica (Huds.) Fr.	o	o	o	×
Flammula alnicola Fr.	o	o	—	—
F. carbonaria Fr.	o	o	o	o
F. gummosa (Lasch.) Fr.	o	o	o	o
F. hybrida Fr.	—	—	o	×
F. lenta (Pers.) Fr.	o	o	o	o
F. lubrica (Pers.) Fr.	—	—	o	o
F. ochrochlora Fr.	—	—	o	o
F. sapinea Fr.	o	×	o	×
F. tricholoma (A. & S.) Fr.	o	o	o	o
Fomes annosus Fr.	o	o	o	o
F. ferruginosus (Schrad.) Massee	o	o	o	o
F. fomentarius (Linn.) Fr.	o	o	—	—
F. igniarius (Linn.) Fr.	o	o	—	—
F. salicinus (Pers.) Fr.	o	o	—	—
F. ulmarius (Sow.) Fr.	—	—	o	o
Galera hypnorum (Schrank.) Fr.	o	×	o	×
G. mniophila (Lasch.) Fr.	o	o	o	o
G. mycenopsis Fr.	—	—	o	o
G. plicatella Peck.	—	—	o	o
G. rubiginosa (Pers.) Fr.	o	o	o	×
G. tenera (Schaeff.) Fr.	—	—	o	o
Ganoderma applanatum (Pers.) Pat.	o	o	o	o
Geaster fimbriatus Fr.	o	o	o	o
G. triplex Jungh.	o	o	—	—
Gomphidium maculatus (Scop.) Fr.	o	o	—	—
G. roseus (Fr.) Quél.	o	o	o	o
G. viscidus (Linn.) Fr.	o	o	o	o
Grandinia farinacea (Pers.) Bourd. & Galz.	o	o	o	o
Gyroporus cyanescens (Bull.) Quél.	—	—	o	o
Hebeloma anthracophilum Maire.	o	×	—	—
H. claviceps Fr.	—	—	o	×
H. crustuliniforme (Bull.) Fr.	o	×	o	×
H. mesophaeum Fr.	o	×	o	×
H. sacchariolens Quél.	o	×	o	o
H. strophosum Fr.	o	×	o	o
H. testaceum (Batsch.) Fr.	o	o	—	—
H. versipelle Fr.	—	—	o	o
Hydnum auriscalpium (Linn.) Fr.	—	—	o	o
H. cirrhatum (Pers.) Fr.	—	—	o	o
H. imbricatum (Linn.) Fr.	o	×	—	—
H. nigrum Fr.	o	o	—	—

TABLE 1 (*cont.*)

	Bact. coli	*Staph. aureus*	*Bact. coli*	*Staph. aureus*
Hydnum repandum (Linn.) Fr.	×	×	o	o
H. zonatum (Batsch.) Fr.	×	×	o	o
Hygrophorus calyptraeformis (Berk.)	–	–	o	o
H. ceraceus (Wulf.) Fr.	o	×	o	o
H. chlorophanus Fr.	o	o	×	o
H. chrysodon Fr.	o	×	–	–
H. cinereus Fr.	o	o	o	o
H. conicus (Scop.) Fr.	o	o	o	o
H. discoideus (Pers.) Fr.	o	o	–	–
H. eburneus (Bull.) Fr.	×	×	×	×
H. fornicatus Fr.	o	o	–	–
H. hypothejus Fr.	o	o	o	o
H. intermedius Pass.	–	–	o	o
H. lacmus Fr.	–	–	o	o
H. laetus (Pers.) Fr.	o	o	o	o
H. miniatus Fr.	o	o	o	o
H. nemoreus (Lasch.) Fr.	×	o	–	–
H. nigrescens Quél.	o	o	o	o
H. nitratus (Pers.) Fr.	o	o	–	–
H. niveus (Scop.) Fr.	o	o	×	×
H. obrusseus Fr.	–	–	o	o
H. olivaceo-albus Fr.	o	o	o	o
H. penarius Fr.	o	o	×	o
H. pratensis (Pers.) Fr.	o	o	o	o
H. psittacinus (Schaeff.) Fr.	o	o	o	o
H. puniceus Fr.	o	o	o	o
H. Reai Maire	o	o	–	–
H. Reai var. *insipida* Lange	–	–	o	o
H. russocoriaceus Berk. & Miller	o	o	o	×
H. subradiatus (Schum.) Fr.	o	o	o	o
H. unguinosus Fr.	o	o	o	o
H. virgineus (Wulf.) Fr.	×	×	o	o
Hymenochaete rubiginosa (Dicks.) Lev.	–	–	o	o
Hypholoma Candolleanum Fr.	o	o	o	o
H. capnoides Fr.	o	o	–	–
H. dispersum Fr.	o	o	o	o
H. fasciculare (Huds.) Fr.	o	o	o	o
H. hydrophilum (Bull.) Fr.	o	o	o	o
H. sublateritium (Schaeff.) Fr.	o	o	o	o
H. velutinum (Pers.) Fr.	o	o	o	o
Inocybe auricoma (Batsch.) Fr.	o	o	o	o
I. Cookei Bres.	–	–	o	o
I. corydalina Quél.	o	o	–	–
I. deglubens Fr.	o	o	o	o
I. descissa Fr.	–	–	o	×
I. fastigiata (Schaeff.) Fr.	o	o	o	o
I. geophylla (Sow.) Fr.	o	o	o	o
I. geophylla var. *lilacina* Fr.	o	o	o	o
I. Godeyi Gillett	–	–	×	×
I. griseo-lilacina Lange	–	–	o	o
I. hystrix Fr.	o	o	–	–
I. incarnata Bres.	×	×	×	×
I. lacera Fr.	o	o	–	–
I. lucifuga Fr.	o	o	–	–
I. maculata Boud.	–	–	o	o
I. obscura (Pers.) Fr.	o	o	o	×
I. pyriodora (Pers.) Fr.	×	×	o	o
I. tomentosa (Jungh.) Quél.	o	o	o	o
I. (*Astrosporina*) *asterospora* Quél.	o	o	o	o
I. (*A.*) *Boltonii* Heim.	o	o	–	–
I. (*A.*) *decipientoides* (Peck) Kühner	o	o	–	–
I. (*A.*) *fulvella* Bres.	o	o	–	–
I. (*A.*) *longicystis* (A. & R.) Kühner = *lanuginosa* Fr. pro parte	o	o	–	–
I. (*A.*) *napipes* Lange	o	o	o	o
I. (*A.*) *ovato-cystis* Kühner = *lanuginosa* Fr. pro parte	o	o	–	–
I. (*A.*) *petiginosa* (Fr.) Rea	–	–	o	o
I. (*A.*) *praetervisa* (Quél.) Schroet.	–	–	o	o
I. (*A.*) *striata* Bres.	o	o	o	o
I. (*A.*) *umbrina* Bres.	o	o	o	o
Irpex obliquus (Schrad.) Fr.	o	o	o	o
I. pachyodon (Pers.) Quél.	–	–	o	o
Laccaria laccata (Scop.) B. & Br.	o	o	o	o
L. laccata var. *amethystina* (Vaill.) B. & Br.	o	o	o	×
L. laccata var. *proxima* (Boud.) Maire	o	o	o	o
L. tortilis (Bolt.) Boud.	o	o	–	–
Lactarius aspideus Fr.	–	–	o	×
L. blennius Fr.	o	o	o	o
L. camphoratus (Bull.) Fr.	o	o	o	o
L. chrysorrheus Fr.	o	×	o	o
L. cimicarius (Batsch.) Cke.	–	–	o	o
L. controversus (Pers.) Fr.	o	o	×	o
L. deliciosus (Linn.) Fr.	o	o	o	o
L. fuliginosus Fr.	–	–	o	o
L. glyciosmus Fr.	o	o	o	o
L. insulsus Fr.	–	–	o	o
L. mitissimus Fr.	o	o	o	o
L. pallidus (Pers.) Fr.	o	×	o	o
L. piperatus (Scop.) Fr.	o	o	o	o
L. piperatus var. *pergamenus* (Swartz.) Quél.	o	o	–	–
L. pubescens Fr.	–	–	o	o
L. pyrogalus (Bull.) Fr.	o	o	o	o
L. quietus Fr.	o	o	×	×
L. rufus (Scop.) Fr.	o	o	o	o
L. scrobiculatus (Scop.) Fr.	–	–	o	o
L. serifluus (DC.) Fr.	o	o	o	o
L. spinosulsus Quél.	o	o	o	o
L. subdulcis (Pers.) Fr.	o	o	o	o
L. theiogalus (Fr.) Plowr.	o	o	o	o
L. torminosus (Schaeff.) Fr.	o	o	o	o
L. trivialis Fr.	o	o	–	–
L. turpis (Weinm.) Fr.	o	o	o	×
L. vellereus Fr.	×	×	×	×
L. vietus Fr.	o	o	o	o
L. volemus Fr.	o	o	o	o
L. zonarius (Bull.) Fr.	–	–	o	o
Lentinus cochleatus (Pers.) Fr.	–	o	–	×
Lenzites betulina (Linn.) Fr.	o	o	o	o

TABLE 1 (*cont.*)

	Bact. coli	*Staph. aureus*	*Bact. coli*	*Staph. aureus*
Lepiota acutesquamosa (Weinm.) Fr.	–	–	o	o
L. alba Bres.	–	–	o	o
L. amianthina (Scop.) Fr.	o	o	–	–
L. brunneo-incarnata Chodat & Martin	o	o	–	–
L. Bucknallii B. & Br.	o	o	o	o
L. carcharias (Pers.) Fr.	o	o	o	o
L. castanea Quél.	o	×	–	–
L. clypeolaria (Bull.) Fr.	o	o	o	o
L. cortinarius Lange	o	×	×	×
L. cristata (A. & S.) Fr.	o	o	o	o
L. excoriata (Schaeff.) Fr.	o	o	–	–
L. felina (Pers.) Fr.	o	o	–	–
L. fulvella Rea	o	o	–	–
L. granulosa (Batsch.) Fr.	o	o	o	o
L. irrorata Quél.	o	o	o	o
L. lenticularis (Lasch.) Cke.	o	o	–	–
L. mastoidea Fr.	o	o	o	o
L. naucina Fr.	–	–	×	o
L. procera (Scop.) Fr.	o	o	o	o
L. rhacodes (Vitt.) Fr.	×	o	o	o
L. sistrata Fr.	o	o	o	o
Leptonia asprella Fr.	o	o	–	–
L. formosa Fr.	o	o	–	–
L. lampropus Fr.	o	×	o	×
L. sarcita (Fr.) Quél.	o	o	–	–
L. sericella (Fr.) Quél.	o	o	o	o
L. serrulata (Pers.) Fr.	o	o	–	–
Lycoperdon caelatum (Bull.) Fr.	o	o	o	o
L. depressum Bon.	o	o	o	o
L. echinatum Pers.	o	o	–	–
L. excipuliforme (Desmaz.)	–	–	o	o
L. giganteum (Batsch.) Pers.	–	–	×	o
L. perlatum Pers.	o	o	o	o
L. pusillum (Batsch.) Pers.	o	o	–	–
L. pyriforme (Schaeff.) Pers.	o	o	o	o
L. saccatum (Vahl.) Fr.	o	×	o	o
L. umbrinum Pers.	o	o	o	o
Marasmius alliaceus (Jacq.) Fr.	×	×	×	×
M. cohaerens (A. & S.) Cke.	o	×	–	–
M. conigenus (Pers.) Karst.	o	o	o	×
M. dryophilus (Bull.) Karst.	o	o	o	o
M. erythropus (Pers.) Fr.	–	–	o	o
M. esculentus (Wulf.) Karst.	–	–	o	o
M. foetidus (Sow.) Fr.	–	–	×	×
M. fuscopurpureus (Pers.) Fr.	×	o	–	–
M. globularis Fr.	o	o	o	o
M. hariolorum (DC.) Quél.	o	o	o	o
M. impudicus Fr.	o	o	o	o
M. oreades (Bolt.) Fr.	o	o	o	o
M. peronatus (Bolt.) Fr.	o	o	o	o
M. ramealis (Bull.) Fr.	–	–	o	o
Merulius corium (Pers.) Fr.	–	–	o	o
M. hymentioides Fr.	–	–	o	×
M. lacrymans (Wulf.) Fr.	o	o	–	–
M. laeticolor B. & Br.	–	–	×	×
M. rufescens (Pers.) Fr.	–	–	o	×
M. tremellosus (Schrad.) Fr.	o	o	o	o
Mycena aetites Fr.	o	o	o	o
M. amicta Fr.	–	–	o	o
M. ammoniaca Fr.	o	o	o	o
M. cinerella Karst.	o	×	o	×
M. epipterygia (Scop.) Fr.	o	o	o	o
M. epipterygioides Pearson	o	o	o	o
M. fellea Lange	o	o	o	o
M. filopes (Bull.) Fr.	o	o	–	–
M. flavoalba Fr.	–	–	o	o
M. galericulata (Scop.) Fr.	o	×	o	×
M. galopus (Pers.) Fr.	o	o	o	o
M. hiemalis (Osbeck.) Fr.	o	o	–	–
M. inclinata Fr.	o	o	o	o
M. lactea (Pers.) Fr.	–	–	o	o
M. metata Fr.	o	o	o	o
M. pelianthina Fr.	o	o	o	o
M. polygramma (Bull.) Fr.	o	o	–	–
M. pura (Pers.) Fr.	o	o	o	o
M. pura var. *carnea* Rea	o	o	o	o
M. rubro-marginata Fr.	o	o	o	o
M. sanguinolenta (A. & S.) Fr.	–	–	o	o
M. uracea Pearson	×	×	×	×
M. viscosa (Secr.) Maire	o	o	–	–
M. vitilis Fr.	o	o	o	o
Naucoria centunculus Fr.	–	–	o	o
N. Cucumis (Pers.) Fr.	o	o	o	o
N. escharoides Fr.	o	o	o	o
N. semiorbicularis (Bull.) Fr.	–	–	o	o
N. siparia Fr.	–	–	o	o
N. submelinoides var. *alnetorum* Maire	–	–	o	o
Nolanea cetrata (Fr.) Schroet.	o	o	o	o
N. papillata Bres.	o	o	o	o
N. staurospora Bres.	o	o	o	o
N. strigosissima Rea	–	–	o	o
Nyctalis asterophora Fr.	o	o	o	o
N. parasitica (Bull.) Fr.	o	o	o	o
Odontia bicolor (A. & S.) Bres.	–	–	o	o
Omphalia fibula (Bull.) Fr.	o	×	–	–
O. hepatica (Batsch.) Fr.	o	×	o	o
O. hydrogramma (Bull.) Fr.	o	o	o	×
O. maura Fr.	o	×	o	×
O. muralis (Sow.) Fr.	o	o	–	–
O. rosella J. E. Lange	–	–	o	o
O. rustica Fr.	–	–	o	o
O. umbilicata (Schaeff.) Fr.	o	o	–	–
Panaeolus papilionaceus (Bull.) Fr.	o	o	o	o
P. sphinctrinus Fr.	–	–	o	o
P. subbalteatus B. & Br.	o	o	–	–
Panus stipticus (Bull.) Fr.	–	–	o	×
P. torulosus (Pers.) Fr.	–	–	o	×
Paxillus atrotomentosus (Batsch.) Fr.	o	o	o	o
P. involutus (Batsch.) Fr.	o	o	o	o
P. panuoides Fr.	o	o	o	o
P. paradoxus (Kalchbr.) Quél.	o	o	o	o
Peniophora cinerea (Fr.) Cke.	–	–	o	o
P. gigantea (Fr.) Massee	o	×	o	×

TABLE 1 (*cont.*)

	Bact. coli	*Staph. aureus*	*Bact. coli*	*Staph. aureus*
Peniophora laevigata (Fr.) Massee	–	–	o	o
P. pallidula (Bres.) Boud. & Galz.	–	–	o	o
P. pubera (Fr.) Sacc.	–	–	o	×
P. quercina (Pers.) Cke.	o	o	o	o
P. setigera (Fr.) Bres.	o	o	o	×
P. velutina (DC.) Cke.	–	–	o	o
Phallus impudicus (Linn.) Pers.	o	o	o	o
Phlebia merismoides Fr.	o	o	o	o
Pholiota aegerita (Porta.) Fr.	–	–	o	o
P. aurea (Mattusch.) Fr.	–	–	o	o
P. aurivella (Batsch.) Fr.	o	o	o	×
P. lucifera (Lasch.) Fr.	–	–	o	o
P. marginata (Batsch.) Fr.	o	o	o	o
P. mutabilis (Schaeff.) Fr.	o	o	o	o
P. praecox (Pers.) Fr.	–	–	o	o
P. pumila Fr.	–	–	o	o
P. radicosa (Bull.) Fr.	–	–	o	×
P. spectabilis Fr.	o	×	o	×
P. squarrosa (Bull.) Fr.	o	o	o	o
P. togularis (Bull.) Fr.	×	×	–	–
Phylacteria anthocephala (Bull.) Fr.	o	o	o	o
P. terrestris (Ehrh.) Big. & Guill.	–	–	o	o
Pleurotus dryinus (Pers.) Fr.	–	–	o	o
P. ostreatus (Jacq.) Fr.	o	o	o	o
P. palmatus (Bull.) Quél.	–	–	o	×
P. petaloides (Bull.) Fr.	–	–	o	o
P. sapidus Schulz.	–	–	o	×
Pluteus cervinus (Schaeff.) Fr.	o	o	o	o
P. eximeus Saund. & Sm.	–	–	o	o
P. nanus (Pers.) Fr.	–	–	o	o
P. nanus var. *lutescens* Fr.	o	o	–	–
P. patrichius Schulz.	–	–	o	o
P. salicinus (Pers.) Fr.	–	–	o	o
Polyporus adustus (Willd.) Fr.	o	o	o	×
P. amorphous Fr.	–	–	o	×
P. benzoinus (Wahlenb.) Fr.	o	o	o	o
P. betulinus (Bull.) Fr.	o	×	o	×
P. caesius (Schrad.) Fr.	o	o	o	o
P. fragilis Fr.	o	×	–	–
P. giganteus (Pers.) Fr.	o	o	o	o
P. hispidus (Bull.) Fr.	–	–	o	o
P. intybaceus Fr.	–	–	o	o
P. perennis (Linn.) Fr.	o	o	o	o
P. radiatus (Sow.) Fr.	o	o	×	o
P. Schweinitzii Fr.	o	o	o	o
P. squamosus (Huds.) Fr.	o	o	o	o
P. stipticus (Pers.) Fr.	–	–	o	o
P. sulphureus (Bull.) Fr.	o	o	o	×
P. tephroleucus Fr.	o	×	o	o
Polystictus abietinus (Dicks.) Fr.	o	o	o	o
P. versicolor (Linn.) Fr.	o	×	o	×
Poria hymenocystis (A. & S.) Fr.	–	–	o	o
P. sanguinolenta (A. & S.) Fr.	–	–	o	o
Psaliota amethystina Quél.	o	o	o	o
P. arvensis (Schaeff.) Fr.	×	×	×	×
P. augusta Fr.	–	–	o	o
P. campestris (Linn.) Fr.	o	o	o	o
P. comtula Fr.	o	o	o	o
P. haemorrhoidaria Kalchbr.	o	o	o	o
P. sylvatica (Schaeff.) Fr.	–	–	o	o
P. sylvicola (Vitt.) Fr.	o	o	o	o
P. villatica (Boud.) Bres.	–	–	o	o
P. xanthoderma var. *leipotoides* Maire	×	×	×	×
P. xanthoderma var. *obscurata* Maire	–	–	×	×
Psathyra conopilea Fr.	o	o	–	–
P. corrugis (Pers.) Fr.	o	o	–	–
P. fibrillosa (Pers.) Fr.	–	–	o	o
P. gossypina (Bull.) Fr.	o	o	–	–
P. obtusata Fr.	–	–	o	o
P. pennata Fr.	o	o	o	o
P. spadiceo-grisea Fr.	–	–	o	o
Psathyrella atomata Fr.	o	o	o	o
P. crenata (Lasch.) Fr.	o	o	–	–
P. disseminata (Pers.) Fr.	o	o	o	o
P. gracilis Fr.	o	o	o	o
Psilocybe foenisecii (Pers.) Fr.	o	o	o	o
P. physaloides (Bull.) Fr.	–	–	o	×
P. Polytrichi Fr.	o	o	–	–
P. sarcocephala Fr.	–	–	o	o
P. semilanceata Fr.	o	o	o	o
P. spadicea Fr.	–	–	o	o
P. subericaea Fr.	o	o	o	o
Ptychogaster albus Cda.	o	o	–	–
Russula adusta (Pers.) Fr.	o	o	o	o
R. alutacea (Pers.) Fr.	–	–	o	o
R. atropurpurea (Krombh.) Maire	o	o	o	o
R. azurea Bres.	o	o	o	o
R. brunneo-violacea Crawshay	o	o	–	–
R. caerulea Cke.	o	o	o	o
R. claroflava Grove	o	o	o	o
R. cyanoxantha (Schaeff.) Fr.	o	o	o	o
R. delica Fr.	o	o	–	–
R. densifolia (Secr.) Gill.	o	o	o	o
R. drimeia Cke.	o	o	o	o
R. emetica (Schaeff.) Fr.	o	o	o	o
R. fallax Fr.	o	o	o	o
R. farinipes Romell	o	o	o	o
R. fellea Fr.	o	o	o	o
R. foetens (Pers.) Fr.	o	o	o	o
R. fragilis (Pers.) Fr.	o	o	o	o
R. graminicolor (Secr.) Quél.	–	–	o	o
R. grisea (Pers.) Bres.	o	o	o	o
R. heterophylla Fr.	o	o	o	o
R. laurocerasi Melzer	o	o	–	–
R. lutea (Huds.) Fr.	o	o	o	o
R. luteotacta Rea	–	–	o	o
R. maculata Quél.	–	–	o	o
R. mustelina Fr.	–	–	o	o
R. nauseosa (Pers.) Fr.	–	–	o	o
R. nigricans (Bull.) Fr.	o	o	o	o
R. ochroleuca (Pers.) Fr.	o	o	o	o

TABLE 1 (*cont.*)

	Bact. coli	*Staph. aureus*	*Bact. coli*	*Staph. aureus*
Russula pectinata (Bull.) Fr.	o	o	o	o
R. pseudointegra Azn. & Gor.	—	—	o	o
R. puellaris Fr.	o	o	—	—
R. punctata (Gill.) Maire	—	—	o	o
R. Queletii (Fr.) Bataille	o	o	o	o
R. rosacea (Pers.) Fr.	—	—	o	o
R. rosea (Schaeff.) Quél.	o	o	o	o
R. solaris Ferd. & Winge	o	o	—	—
R. Velenovskyi Melzer & Zvara	o	o	o	o
R. venosa (Vel.) Melzer	o	o	o	o
R. versicolor Schaeffer	o	o	o	o
R. vesca Fr.	o	o	o	o
R. virescens (Schaeff.) Fr.	o	o	o	o
R. xerampelina (Schaeff.) Fr.	o	o	o	o
R. xerampelina var. *fusca* Quél.	—	—	o	o
Schizophyllum commune Fr.	o	o	o	o
Scleroderma aurantium Pers.	o	o	o	o
S. verrucosum (Vaill.) Pers.	o	o	o	o
Sebacina incrustans (Pers.) Tul.	o	o	—	—
Sparassis crispa (Wulf.) Fr.	o	o	o	o
S. laminosa Fr.	—	—	o	o
Sphaerobolus stellatus (Tode) Pers.	—	—	o	o
Stereum hirsutum (Willd.) Fr.	o	o	o	o
S. purpureum (Pers.) Fr.	o	o	o	o
S. rugosum (Pers.) Fr.	—	—	o	o
S. sanguinolentum (A. & S.) Fr.	o	×	o	o
S. spadiceum Fr.	o	o	o	o
Stropharia aeruginosa (Curt.) Fr.	o	×	o	×
S. albocyanea (Desm.) Fr.	o	o	o	o
S. depillata (Pers.) Fr.	—	—	o	o
S. semiglobata (Batsch.) Fr.	o	o	o	o
S. squamosa (Pers.) Fr.	o	o	o	o
Trametes gibbosa (Pers.) Fr.	o	o	o	o
T. mollis (Sommerf.) Fr.	—	—	o	o
T. rubescens (A. & S.) Fr.	o	o	o	o
Tremella foliacea (Pers.) Fr.	—	—	o	o
T. mesenterica (Retz.) Fr.	o	o	o	o
Tremellodon gelatinosum (Scop.) Pers.	—	—	o	×
Tricholoma acerbum (Bull.)Fr.	—	—	o	o
T. albobrunneum (Pers.) Fr.	o	×	o	×
T. album (Schaeff.) Fr.	—	—	o	o
T. argyraceum (Bull.) Fr.	o	o	o	o
T. atrosquamosum (Chev.) Sacc.	o	o	—	—
T. aurantium (Schaeff.) Fr.	o	o	o	o
T. bufonium (Pers.) Fr.	o	o	o	o
T. carneum (Bull.) Fr.	o	o	o	o
T. cinerascens (Bull.) Konrad	o	o	o	o
T. cognatum Fr.	o	o	—	—
T. columbetta Fr.	o	o	o	o
T. cuneifolium Fr.	o	o	o	o
T. equestre (Linn.) Fr.	—	—	o	o
T. fulvum (DC.) Fr.	o	o	o	o
T. gambosum Fr.	—	—	o	o
T. grammopodium (Bull.) Fr.	o	o	o	o
T. imbricatum Fr.	o	×	o	×
T. irinum Fr.	×	×	×	×
T. lascivum Fr.	o	o	o	o
T. leucocephalum Fr.	o	o	o	o
T. melaleucum (Pers.) Fr.	o	o	o	o
T. murinaceum (Bull.) Fr.	o	o	—	—
T. nudum (Bull.) Fr.	o	o	×	×
T. panaeolum Fr.	×	×	×	×
T. personatum Fr.	×	×	×	×
T. polioleucum Fr.	o	o	—	—
T. portentosum Fr.	o	o	o	o
T. psammopum Kalchbr.	—	—	×	×
T. putidum Fr.	o	o	—	—
T. rutilans (Schaeff.) Fr.	o	×	×	×
T. saponaceum Fr.	×	×	×	×
T. sejunctum (Sow.) Fr.	—	—	o	o
T. sordidum (Schum.) Fr.	o	×	o	o
T. sulphureum (Bull.) Fr.	o	o	o	o
T. terreum (Schaeff.) Fr.	o	o	o	o
T. truncatum (Schaeff). Quél.	—	—	o	o
T. ustale Fr.	o	×	o	×
T. virgatum Fr.	o	o	—	—
Tubaria autochthona (B. & Br.) Sm.	—	—	o	o
T. furfuracea (Pers.) W. G. Sm.	o	o	o	o
T. paludosa Fr.	o	o	—	—
T. pellucida (Bull.) Fr.	o	o	—	—
Tylopilus felleus (Bull.) Karst.	o	o	o	o
Volvaria bombycina (Schaeff.) Fr.	—	—	o	o
V. speciosa Fr.	—	—	o	o

A. virosa, *Clitocybe inversa*, *Lepiota cortinarius*, and *Omphalia fibula*, showed any reaction against *Pseudomonas pyocyanea* the results against that bacterium are omitted from the list. No test is indicated by the symbol '—', a positive result by '×' and a negative result by 'o'. The positive symbol is given only to those extracts which gave a zone of at least 2 mm. in width by the above-mentioned method, but, as the degree of positive reaction is not indicated, × may represent a very slight or a very pronounced bacteriostatic action.

A detailed analysis of Table 1 would serve no useful purpose; but it is apparent that a considerable number of the sporophores tested gives a positive reaction against one or both of the bacteria. Up to date we could state that approximately fifty species give a strong positive reaction and at least ten species are outstandingly good.

CULTURAL WORK

As the sporophores of these woodland and pasture types are seasonal and often infrequent it is desirable that they should be isolated in pure culture so that if, under cultural conditions, they still form bac-

teriostatic substances, these can be produced when required.

It is first necessary to establish a relationship between the type of reaction obtained from the sporophore extract and the type of reaction obtained from the metabolism solution produced by the fungus in culture, e.g. does a positive sporophore extract always give a positive metabolism solution and a negative sporophore extract invariably give a negative metabolism solution, because if so only species whose sporophore extract is positive need be cultured? In order to test this about thirty species have been chosen at random from those listed in Table 1. These, which include both positives and negatives, have been grown in culture and a comparison has been made between the antibacterial effect of the sporophore extract and that of the subsequent metabolism solution for each species. Two methods have been employed for growing and testing:

(1) The fungus is grown on an agar medium, usually malt agar, and when sufficiently large, disks of mycelium and agar 11 mm. diam. are cut from the colony with a sterile cork borer. These are put on to the surface of 10 ml. of a liquid medium contained in 50 ml. flasks. As the amount of bacteriostatic substance produced is invariably less from submerged mycelium the disk must float; this can be ensured by using, when necessary, cork disks the same size as the inoculum. After the culture has been growing for about 20 days (this period is extremely variable) the metabolism solution is tested for bacteriostatic action by the method which has already been briefly described.

(2) The second method is more rapid but limited in application (Wilkins & Harris, 1944*a*). Disks of mycelium and agar are again cut from a growing colony. Each disk is dropped into the centre of a plate of bacteria-incorporated agar medium while the latter is still liquid. The plate is incubated at 37° C. overnight. If bacteria-inhibiting substances are produced, the following morning there will be a clear, bacteria-free zone round the edge of the colony. As the disks are of uniform size, and as the fungus will not grow at 37° C., the width of the zone will indicate the relative potency of the bacteriostatic substances produced by different fungi.

Table 2 shows the relation between the bacteriostatic action of (*a*) sporophore extract, (*b*) mycelial disk and (*c*) metabolism solution, in each case against *Bact. coli* and *Staph. aureus*, for twelve representative fungi chosen from the above-mentioned thirty isolations.

Within the limited experience gained from experimental work on the thirty species and a number of strains of these species, we can make the following generalizations as to the relation between the bacteriostatic action of sporophore extract and metabolism solution as illustrated by Table 2:

(1) A fungus which gives a totally negative result in sporophore extract generally gives a totally negative result in metabolism solution, e.g. *Armillaria mellea*, *Clitocybe nebularis* and *Lepiota rachodes*. It may give a positive result against *Staph. aureus* only, e.g. *Pholiota aegerita*, or it may give a positive result against both *Bact. coli* and *Staph. aureus*, e.g. *Coprinus picaceus*, but no case is known where it gives a positive result against *Bact. coli* only.

(2) A fungus which gives a positive result against one of the two bacteria generally gives a positive result in culture, but again this is either against *Staph. aureus* only, e.g. *Clitocybe aurantiaca* and *Clitocybe clavipes*, or against both, e.g. *Marasmius conigenus*. Here, also, there is no evidence of a positive result against *Bact. coli* only, even if the sporophore extract acted against *Bact. coli* only, e.g. *Clitocybe aurantiaca*.

(3) A fungus whose sporophore extract gives a positive result against both bacteria generally acts against both bacteria in metabolism solution, e.g. *Clitocybe inversa*, *Psalliota arvensis* and *Tricholoma*

TABLE 2. *Bacteriostatic relations of twelve representative fungi*

	Sporophore extract		Mycelial disk		Culture liquid	
	Bact. coli	*Staph. aureus*	*Bact. coli*	*Staph. aureus*	*Bact. coli*	*Staph. aureus*
Armillaria mucida	o	o	o	o	o	o
Clitocybe aurantiaca	×	o	o	×	o	×
C. clavipes	o	×	o	×	o	×
C. inversa	×	×	×	×	×	×
C. nebularis	o	o	o	o	o	o
Coprinus picacèus	o	o	×	×	×	×
Lepiota rachodes	o	o	o	o	o	o
Marasmius conigenus	o	×	×	×	×	×
Pholiota aegerita	o	o	o	×	o	×
Psaliota arvensis	×	×	×	×	×	×
Tricholoma nudum	×	×	×	×	×	×
T. personatum	×	×	o	×	o	×

nudum. It may act against *Staph. aureus* only (usually only on certain media), e.g. *Tricholoma personatum* but never against *Bact. coli* only.

From the above it is obvious that most of the positive results are against both *Staph. aureus* and *Bact. coli* or against *Staph. aureus* only but never against *Bact. coli* only.

By reference to Table 2 the importance of the mycelial-disk method is shown to be that it anticipates exactly what result may be expected from the metabolism solution, whereas, except in the wholly positive or wholly negative sporophore extracts (and not always then!), there is no direct relation between the results of the sporophore extract and the metabolism solution. If this function of the mycelial-disk method can be taken as established, the employment of the method will shorten the experimental time by about a month, so ensuring a considerable economy in both time and material.

Two major factors tend to complicate the work. One is that a negative sporophore extract is no criterion that the same fungus in culture will also be negative though, as this usually is the case, these fungi are naturally regarded as of less significance. The other is that sporophore extracts of different individuals (? strains) of the same species may give quite opposite results, some positive in varying degrees and some negative. This seems to be due to (1) age of the sporophore, e.g. *Cortinarius collinitus* and *Psalliota arvensis*, or to (2) habitat, e.g. *Hygrophorus eburneus*, *Lactarius vellereus* and *Tricholoma rutilans*, but may be due to other causes not yet discovered. This is being investigated.

Though we fully appreciate the incompleteness of the work, we are of the opinion that, with regard to the production of bacteriostatic substances, the larger Basidiomycetes are extremely promising. The work is being continued.

The number of people who have furthered this investigation is too great for them all to be mentioned, and we hope that omission of names will not be taken as indicative of lack of appreciation on our part. We should find it difficult adequately to express our thanks for the magnificent work done by Mr E. W. Swanton, Curator of the Haslemere Educational Museum and by Mr A. A. Pearson, Treasurer of the British Mycological Society. These two eminent mycologists, by giving most generously of their time and knowledge, have increased the scope of the investigation far beyond what would otherwise have been possible. The senior author is indebted to the Nuffield Research Fund for a grant enabling him to have the valuable help of Dr G. C. M. Harris as Research Assistant and to the Air Ministry for releasing him for that purpose. We are most appreciative of the help in secretarial and field work given by Miss R. M. Forster of Westfield College and by Mrs W. H. Wilkins, the wife of the senior author. Our best thanks for technical assistance are also due to Miss R. Wyburn-Mason and to Mr A. H. Cripps.

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Observations on the method of transmission of internal boll disease of cotton by the cotton stainer-bug

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(With Plate 10, 5 Text-figures and 2 Graphs)

Results are presented to show that all the rules of proof for insect transmission of *Nematospora* by *Dysdercus* spp. have now been satisfied: viz. a close association of the insect with diseased plants in nature; visitation of healthy plants by the insect under conditions suitable for transmission of the disease; the presence of the pathogen in or on the insect in nature or following visitation to a diseased plant; and experimental production of the disease by insect visitation under controlled conditions. Hence it is claimed that *Dysdercus* spp. are proved vectors of internal boll disease of cotton.

Fungal material as spores or mycelium is carried as an external contaminant on the mouth-parts, although it is located in the deep stylet pouches, where protection is afforded and spore germination is possible.

The fungus is cast off with the exuviae during moulting, but recontamination from exuviae or other sources in the environment occurs.

The transmission is hence purely mechanical with the insect obligatory only in its function as a syringe to inject the fungus which is otherwise unable to reach its substrate. Insect and fungus can develop independently and the insect is apparently unharmed by the presence of the fungus.

Nematospora spores also reach the intestine but do not appear to remain viable there and do not germinate.

The fungus is believed to enter and leave the insect by the same route. The spores are well adapted in shape to reach the stylet pouches of the insect.

No alternative morphological form of *Nematospora gossypii* within the insect has been found.

The results do not lead to methods of control of the disease beyond those in practice—trapping of insects, destruction of alternative host plants, and the alteration of time of planting to avoid insect attack.

I. Introduction

Internal boll disease of cotton is caused by *Nematospora gossypii* and allied fungi. It is disseminated by hemipterous insects, especially *Dysdercus* spp. The insect appears to be a vector but the method of transmission is not known: the present investigation is concerned with the mechanism of this transmission. Information which might lead to a solution of the problem was sought along the following lines: (1) by microscopic examination of insects which had been exposed to *Nematospora*-infected material; (2) by reisolation of the fungus from different parts of insects thought to be infected; (3) by testing the infectivity of insects known to have had access to *Nematospora* cultures. The possibility of the occurrence of some unknown morphological form of the fungus inside the insect was also considered.

As the insect transmission of fungal, bacterial and virus diseases of plants is of fairly general occurrence (Leach, 1940), the successful pursuit of the present investigation may be deemed to possess a more general, as well as a specific biological significance.

II. Review of previous literature

Description of the disease. The symptoms of internal boll disease differ from those of other infections of the cotton capsule (e.g. in the rot due to *Xanthomonas malvacearum*, *Glomerella gossypii*, etc.), in that only the lint and seeds are affected and there is no external evidence of decay. In the past, the name has been used to include damage to cotton bolls caused by the attack of hemipterous insects which puncture the wall of the fruit and feed on the developing lint and seeds. Such insect attack alone may cause considerable loss, since young bolls after puncturing are often shed (Pomeroy, 1924), while seeds which have been attacked often fail to develop. A distinction is made, however, between these direct effects of insect injury, and the widespread damage due to the further infestation of the boll contents by parasitic organisms which appear to gain access solely through the insect punctures. In this more exact sense, internal boll disease may be caused by bacteria, but is more often due to one or more of the *Nematospora* group of fungi, responsible also for 'stigmatomycosis' diseases of many other fruits.

The fungus. The genus *Nematospora* was originally founded by Peglion (1901) who described the fungus *N. coryli* from hazel-nuts in 1897, but Borzi (1888) had previously isolated the allied *Eremothecium cymbalariae* from capsules of *Linaria cymbalaria*. Ashby & Nowell (1926) added two more species, *Nematospora gossypii* and *Spermophthora gossypii*, from cotton, and they included under *Nematospora coryli* two others, *N. phaseoli* (Wingard, 1922, 1925) and *N. lycopersici* (Schneider, 1916, 1917). Since 1926, there have been two further additions to the

list, *N. nagpuri* (Dastur & Singh, 1930) and *Eremothecium ashbyi* (Guilliermond, 1936). Marsh (1926) established that, apart from the insect, *Nematospora* is a wound parasite of cotton bolls.

Association of fungus with Dysdercus. Nowell (1917*a*,*b*, 1918) was the first to observe that the disease was always associated with insect punctures, particularly those of *Dysdercus*, the cotton-stainer bug; increasing numbers of infected bolls as stainers became more abundant during the course of the fruiting season, indicated the significance of stainers in spreading the disease in the West Indies (Nowell, 1924). Internal boll disease in Burma was also associated with the prevalence of *D. cingulatus* (Rhind, 1927), and in Fiji with *Dysdercus* spp. (Simmonds, 1929).

Other insects associated with stigmatomycosis. These include *Nezara viridula*, *Leptoglossus bolteatus*, *Oxycarenus* sp., *Homoeocerus* sp., *Tectocoris lineola*, *Chlorochroa sayi* and *Callidea dregii* on cotton; *Nezara hilaris* on Lima bean; *Leptoglossus* sp., *Rhynchocoris serratus* and *Cappaea taprobanensis* on citrus; *Phthia picta* on tomato; *Antestia lineaticollis* on coffee; and *Odontopus confusus* on *Sterculia*.

Evidence that the insect is a vector. Laycock (1925) found that stainers bred under sterile conditions did not produce infections but that those from the field introduced the disease to bolls which had been previously surface-sterilized; Hansford (1929*a*,*b*) failed to isolate *Nematospora* from the surface of bolls under natural conditions and concluded that the fungus must be insect-borne; in experiments with caged plants and controlled insects, Moore (1930) found no infections apart from those which started immediately under insect punctures; and Wallace (1931) showed that individuals of the coffee bug, *Antestia lineaticollis*, from a diseased area could introduce *Nematospora coryli* to a plantation free from the disease.

Nature of the association between insect and fungus. Pearson (1935–6) favoured the view that transmission from boll to boll is purely mechanical, i.e. by contamination of the mouth-parts. His experiments showed that all ages of insect were equally able to cause infections (apart from 2nd stage nymphs, where results were inconclusive because the mortality rate was high) but that there was a strong significant difference in ability of insects to infect before and after moulting. A biological relationship would result in the insects remaining infective for life. Hopkins (1932) also concluded that the fungus must be carried externally on the insect where it would be vulnerable to drought, since punctures were largely sterile when stainers returned to the cotton crop during dry weather. Müller (1939), on the other hand, states that with *N. coryli* infection of citrus, individuals of *Rhynchocoris serratus* and *Cappaea taprobanensis* remain infective for life, even if they only have access to infective material once as newly hatched larvae. This implies a more intimate connexion between insect and fungus.

Presence of the fungus in infective insects. Moore (1930) reports that she demonstrated the 'presence of *Nematospora* alive in the mouth-parts and digestive tract of stainers by dissection and culturing', but she gives no further details. Laycock (1925), Hansford (1930), Nowell (1939), Fawcett (1929) and Müller (1939) all obtained negative results from examination of sections of infective individuals, or from attempts to reisolate the fungus from such insects.

Ecological observations. At the end of the season, adult stainers leave the cotton fields and migrate to alternative host plants, a sequence of which is necessary in some localities before the stainers return to the succeeding cotton crop. The state of development and distribution of these plants will affect the time and numbers of stainers returning to the cotton and the amount of breeding which has occurred in the interval (Ballard, 1927; Bebbington & Allan, 1931–2, 1934–5, 1936, 1936–7, 1937–8; Moore, 1930; Callan, 1943; Pearson, 1932–3, 1937–8; Peat, 1934–5; Krug, 1936; and many others). Many of the alternative host plants of the stainer appear in the list of plants from which *Nematospora* has been recorded (Wallace, 1939), so that there may often be a ready potential source of *Nematospora* from which stainers returning to the cotton would carry the initial inoculum. In support of this, Pearson (1934–5) records that 13 % of the stainers taken from certain *Sterculia* trees were infective, and that another plant-bug, *Odontopus confusus*, which lives entirely on the *Sterculia* was also infective, so that the disease could here be perpetuated independent of stainers.

Detailed knowledge of the mechanism of the transmission of *Nematospora* will add to the value of such data. The absence in nature of the fungus and of insects which transmit it, favours the undertaking of observations on transmission in a district remote from cotton fields.

III. Materials

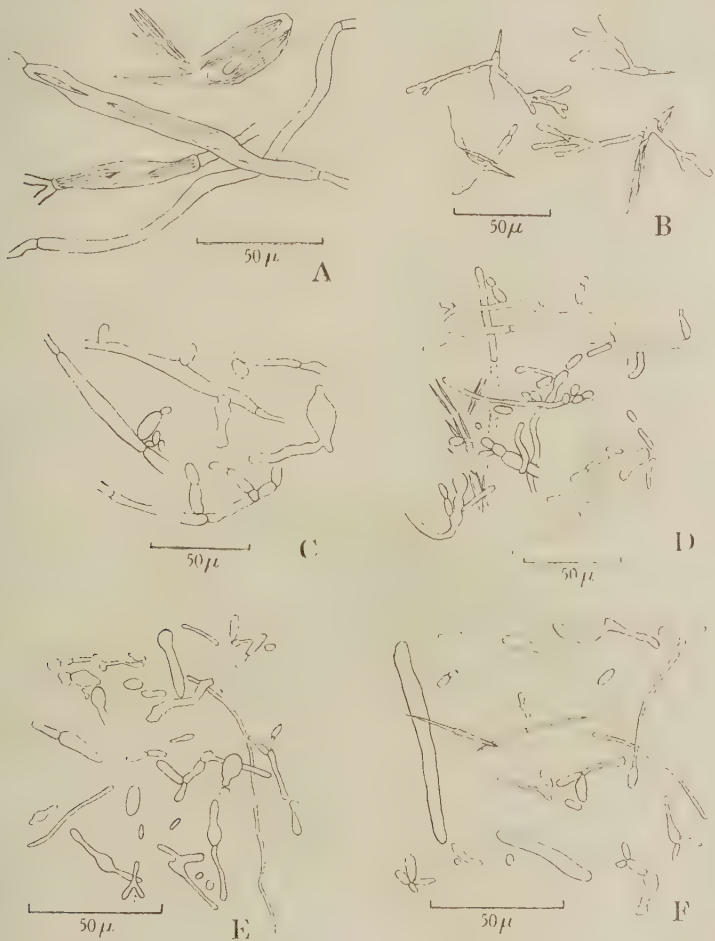
(*a*) *The insect*

The insects used were mainly of a strain of *Dysdercus intermedius*, an African species of stainer-bug kindly supplied to me by Dr C. Potter, of Rothamsted Experimental Station. They were reared in deep Petri dishes containing moistened sand, a cork and cotton seed. The cotton seed used was at least 5 years old, and no evidence of *Nematospora* has ever been found in it. Marsh (1925) was unable to isolate any viable *Nematospora* from a sample of seed cotton on its arrival in this country, although it contained spores. Throughout the present investigation there was no evidence to indicate that further precautions were necessary to prevent contamination from this

source. The glass type of container was satisfactory for all stages of nymph, but adults did not copulate until transferred to zinc cages with more ventilation. At night the containers were kept in an incubator at 24° C.; during the day they were placed under artificial light in the laboratory. Under these conditions the average life history was as follows:

	Days
Time for eggs to hatch	7– 9
Duration of 1st instar	3– 4
Duration of 2nd instar	5– 6
Duration of 3rd instar	6– 7
Duration of 4th instar	7– 9
Duration of 5th instar	12–13 for males 13–16 for females

Copulation started about 5 days after the first female appeared, and the adults lived for a few days to several weeks. The containers were frequently changed and sterilized to keep down the growth of moulds. In the early stages of the work, a few observations were carried out using individuals of *D. Howardi*, a West Indian species then being cultured by Miss E. I. MacGill.



Text-fig. 1. A. Developing and mature sporangia of *N. gossypii*. B. Germinating spores of *N. gossypii*. C. Bud formation in *N. gossypii*—old culture on 2 % malt agar. D. Bud formation in *N. gossypii* on plain agar. E. *N. nagpuri* on potato-sucrose agar. F. *N. coryli* on sterilized cotton-seed kernels.

(*b*) *The fungus*

Two strains of *Nematospora gossypii* were available, both of which caused staining when injected by syringe into developing cotton bolls; on re-isolation, cultures of characteristic appearance were

again obtained. Of these one was a mycelial type producing few spores. The other, which displayed greater vigour in culture, produced spores abundantly and was mainly used in these experiments. It grew well on sterilized potato, and on potato-sucrose agar, where a colony 3·5 cm. in diameter was produced in 7 days at 24° C., consisting mainly of spores. *N. gossypii* is one of the more mycelial of the stigmatomycosis fungi. The spores are needle-shaped and divided transversely into a thick-walled and a thin-walled end. The thin-walled end is prolonged into a long non-motile appendage. During germination, the thin-walled part swells out towards the centre, and one or more germ-tubes are sent out (Text-fig. 1 B). These produce a branching dichotomous mycelium, non-septate except for the cutting off of a sequence of elongated sporangia behind the tips of the hyphae. Inside the sporangia, the spores are usually formed in two bundles at opposite ends, and they are liberated by breakdown of the sporangium wall (Text-fig. 1 A).

Cultures of *N. nagpuri* and *N. coryli* were also grown (Text-figs. 1 E, F); these on solid media produce colonies which are yeast-like in appearance. The mycelium becomes broken up into buds of irregular size and shape, the larger of which become sporangia and produce the needle-shaped spores.

(*c*) *The cotton plant*

Cotton plants of several varieties were grown in a heated greenhouse, average temperature 74° F. It was found impracticable to sow the seed before March in Manchester, since plants sown then soon surpassed those started earlier although all had approximately the same temperature conditions. Light appeared to be the limiting factor. The plants were brought on in pots until about 9 in. high and then planted out into the soil. Flowering started about 14 weeks after planting, and a continuous supply of fruits in limited numbers was then available till the end of November. African and American varieties were found to grow best, and most of the tests were carried out using African bolls.

IV. Experimental methods and observations

Observations resulting from the four-fold approach to the problem indicated in the Introduction will now be considered.

(*a*) *Presentation of the fungus to the insect*

The method of presenting the fungus to the insect most closely approximating to conditions in the field, is as infected cotton bolls. As the seasonal limiting of the bolls to summer and autumn, and the difficulty of obtaining non-contaminated inoculations rendered this impracticable as the regular method, other possibilities were explored.

In a first series of experiments, individual delinted cotton seeds were surface-sterilized, placed on moist filter-paper in Petri dishes and inoculated with *N. gossypii* by needle or syringe, in conformity with the observations of Hansford (1929*b*) and Hopkins (1932), that in nature considerable infection of the actual seed occurs in relation to the feeding of stainers after the bolls have opened. Of nineteen lots of seed so treated, a majority showed failure of *Nematospora* spores to germinate, some became contaminated, and in others the seed germinated after a few days. Slightly better results were obtained from the current year's seed, but on the whole this method was considered inadequate and was not therefore adopted.

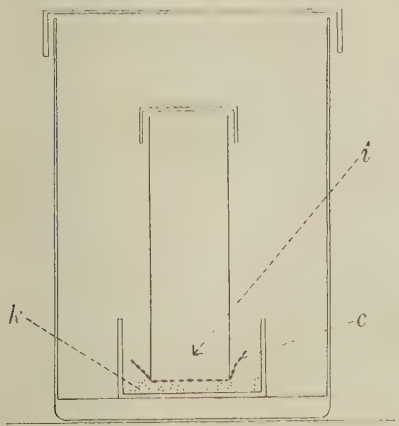
It was observed, however, that *Nematospora* grew well on moistened autoclaved cotton-seed kernels, and that *Dysdercus* would feed fairly readily on this material. The kernels were placed on moist filter-paper in small Petri dishes. In the course of 7 days (at 24° C.) following inoculations, the fungus had grown throughout the dish and mycelium, sporangia and free spores were present. The usual practice adopted was to confine the insects in such cultures for 24 hr. With younger nymphs it was found that a little sand mixed with the cultures reduced the mortality rate.

Presentation of a suspension of *Nematospora* spores in water to the insects was less successful; the spores are difficult to wet and tend to float in clumps on the surface. Nevertheless, some positive results were obtained.

These procedures are both open to the criticism that the entire body of the insect is exposed to the fungus, and, while this may occur in nature when the insects feed on contaminated dehisced fruits, there must be many occasions when only the ends of the stylets make contact with the fungus. It was therefore considered desirable that a method of separating insect and culture should be sought. To this end it was found that when a culture was closely covered with a sheet of cellophane, no spores or mycelium reached the upper side after several days, but insects were able to puncture the cellophane and feed on the culture below; for the younger nymphs a thinner grade of cellophane had to be used. In practice, the cellophane was stretched over perforated porcelain cylinders or across the ends of 3 × 1 in. sections of glass tubing, the insects being trapped inside and the whole being placed over dishes of cotton-seed kernels infected as described above. Each unit was enclosed in a glass container to reduce desiccation and contamination of the culture (Text-fig. 2). As less feeding took place by this method, the period of exposure was increased. When sixty-five insects were treated in this way, *Nematospora* was subsequently detected in the stylet pouches of seventeen and in the intestine of fourteen (see p. 277 and Appendix, Table 1 B). A

number of contaminants grew over the upper surface of the cellophane while the insects were on it. These were examined and found to consist mainly of a yeast which superficially resembled *N. coryli* except for the absence of spores. When subcultured on cotton-seed kernels, however, the sour odour and absence of spores, sporangia or mycelial fragments clearly indicated that an organism other than *Nematospora* was involved. Other contaminants on the cellophane included *Rhizopus*, *Fusarium* and various bacteria; in no case was *Nematospora* seen.

Lastly, a few insects were fed on cotton bolls which had been previously inoculated (on the plant) with *Nematospora* by syringe.



Text-fig. 2. Apparatus used in feeding insects through cellophane. *c*, cellophane; *k*, culture of *Nematospora* on cotton-seed kernels; *i*, position of trapped insects.

(*b*) *Feeding mechanism of* Dysdercus *spp.*

Dysdercus, like other Hemiptera, does not undergo complete metamorphosis during its life history. The individual which hatches from the egg is essentially similar in structure to the adult form, and matures to this by a series of moults during each of which the entire chitinous parts are cast off and replaced by a previously secreted new layer underneath. Hence, the feeding mechanism is similar for all stages, with only an increase in size following each moult. Feeding is by the 'piercing-sucking' type of mouth-parts. The front of the head is prolonged into a long tubular proboscis which is normally folded back to lie horizontally under the body between the three pairs of legs, but is extended vertically downwards when the insect is feeding (Text-figs. 3A, B). The bulk of the proboscis consists of the fleshy labium of four segments; this carries two pairs of stylets in a groove on its dorsal side (i.e. functionally ventral). The outer pair, the mandibles, are barbed and pointed at the distal end, and closely invest the inner pair, the maxillae, which are grooved on the inner surfaces and fit together to form two channels, a dorsal suction canal up which the food passes, and a ventral canal down which the saliva is pumped. The groove of the labium flattens out towards the top, and in this region the proboscis is covered by another shorter flap, the labrum, which then carries the stylets in a groove on its ventral side (Text-fig. 3D). When the insect feeds, the end of the labium remains in contact with the surface of the cotton boll or seed and the surface is punctured by the sharp mandibles. Weber (1930) believes that these are moved forwards alternately and the maxillae follow down between them. As the stylets penetrate deeper, the top two segments of the labium become bent backwards to compensate for the greater length now exposed (Text-fig. 3A). The barbs on the mandibles keep the stylets firmly inserted.

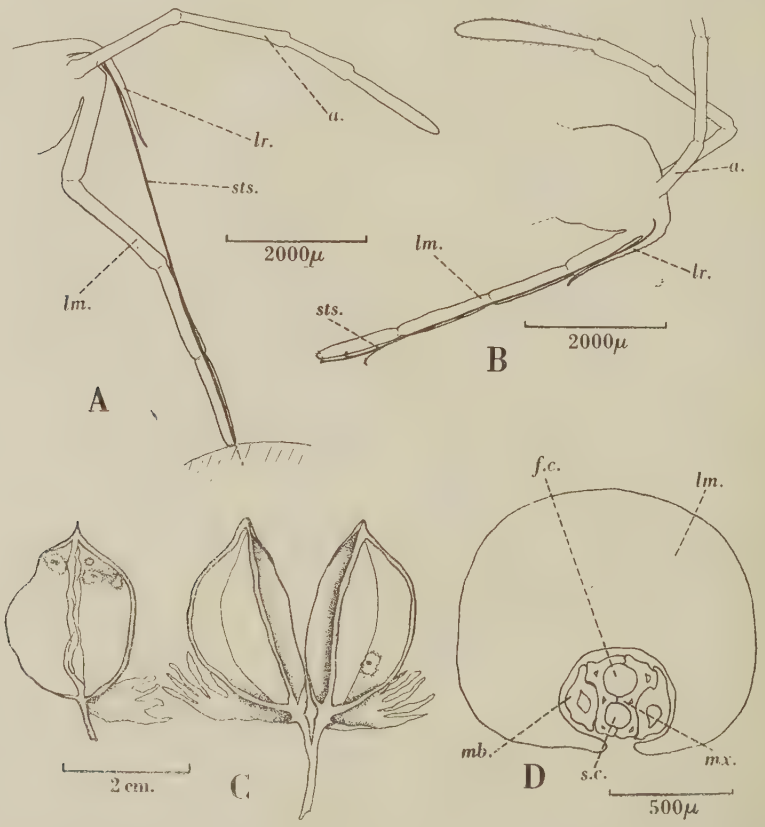
Where the stylets enter the insect head, they separate to right and left (Text-figs. 4, 5A, B); the food channel becomes continuous with the pharynx, and the salivary channel with the exit duct from the salivary pump situated below the pharynx. The stylets diverge farther from one another and broaden out as they extend back, the mandibles terminating slightly in front of the eye and the maxillae almost level with the posterior limit of the eye. Each stylet is lodged in a cavity lined by a differentially thickened layer of chitin continuous with the exoskeleton (Text-figs. 5C, D). In the present account, these invaginations which house the stylets will be termed *stylet pouches*. The thickened inner walls of the maxillary pouches are considered by Snodgrass (1935) to be backward extensions of the hypopharynx and are termed hypopharyngeal plates (Text-fig. 4). Each stylet is articulated by a lever mechanism and is well supplied with muscles enabling it to be moved backwards and forwards in the head.

The pharynx consists of two thickened chitinous plates attached laterally leaving a V-shaped cavity (Text-fig. 5D). During feeding, the saliva is pumped down the salivary channel on to the food by the piston mechanism of the salivary pump. At the same time, the muscles attached to the dorsal wall of the pharynx alternately expand and contract, thus opening and closing the pharynx and setting up a suction which draws the sap and saliva solution up the food channel. During the observations on feeding, it was possible for a short time to watch the ends of the stylets of one insect which had punctured through cellophane; a drop of colourless, transparent fluid—saliva presumably—alternately appeared and disappeared. Tower (1914) found that in *Anasa tristis* (Hemiptera) movement of food up the pharnyx into the oesophagus is controlled by three series of muscles which operate at different times, thus preventing regurgitation of food from the crop. Singh (1923) figures a valve in *Dysdercus cingulatus*, formed by the continuation of the

oesophagus wall into the midgut for about 1 mm. In *D. intermedius* the posterior part of the oesophagus has a mnch-convoluted wall which extends into the midgut aud perhaps fulfils a valvular function. The food passes from the oesophagus to the midgut where absorption takes place, and finally to the

(c) Microscopic examination of contaminated insects before moulting

Adults and all stages of nymph were sectioned after exposure to *Nematospora*-infected material by one of the methods previously described; fixing was



Text-fig. 3. A. Profile of 5th stage nymph head and proboscis when feeding. B. Profile of 4th stage nymph head and proboscis when not feeding. C. Proliferations on inner surface of carpel wall after puncturing by *Dysdercus* (lint and seeds removed). D. Transverse section of labrum and stylets of 4th stage nymph showing food and salivary channels.

Abbreviations to Text-figs 3, 4 and 5. *a*, antenna; *e.ph.* epipharynx; *e.s.d.* exit duct from salivary pump; *f.c.* food channel between maxillae; *h.ph.* hypopharynx; *h.ph.pl.* hypopharyngeal plate (forming inner margin of maxillary stylet pouch); *lm.* labium; *lr.* labrum; *mb.* mandible; *mb.l.* lever to articulate mandible; *mx.* maxilla; *mx.l.* lever to articulate maxilla; *mx.g.d.* maxillary gland ducts; *n.st.s.* sheath from new stylets; *oes.* oesophagus; *ph.* pharynx; *s.* secretion from maxillary glands; *s.c.* salivary channel between maxillae; *s.d.* salivary duct from salivary glands to pump; *s.p.* salivary pump; *st.p.w.* chitinous wall of stylet pouch; *sts.* stylets.

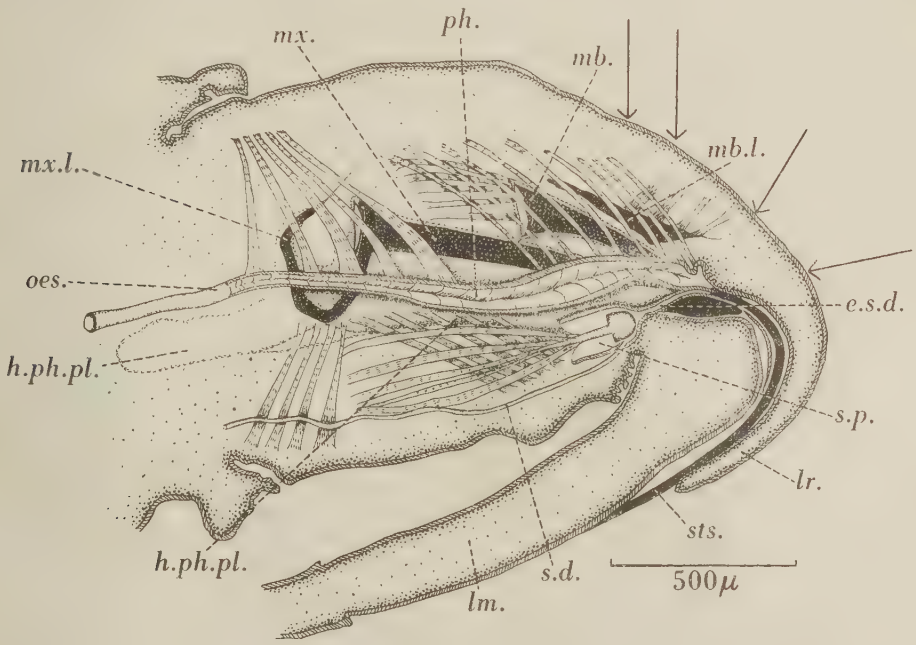
rectum. The excretum of adult insects is a turbid, greenish liquid containing solid material, while that of nymphs is transparent and of lighter colour. The intestine contains numerous thread-like bacteria which are located in groups, particularly near the wall.

carried out either immediately after feeding, or after a lapse of a known number of days. Insects were fixed in acetic alcohol and sectioned from wax (m.p. 53° C.). Various staining methods tried were not successful in differentiating fungus and insect tissues; the fungus spores stain much less rapidly

than insect tissues. By using Delafield's haematoxylin with subsequent mounting in glycerine jelly the spores were more easily identified by diffraction. Mallory's triple stain was useful for insect tissues.

In all, fifty-nine insects were examined after direct exposure to a culture or spore suspension, sixty-five after access through cellophane, and four after feeding on inoculated bolls. The location of the fungus inside the insect was the same with each method of feeding, the only difference being in the percentage of insects contaminated. (For detailed figures see Appendix, Tables 1 A, B, C).

Examination of the insects showed that all except intervened, but only once was a spore observed which was germinating. This spore was present in a 2nd stage nymph fixed immediately after feeding; it was swollen in the centre but no germ tube had developed (Pl. 10, fig. 2). It is possible that this swelling had taken place in the culture dish before the insect fed. In many cases the thin-walled ends of the spores in the intestine appeared shrunken as though partly digested. The spores were frequently located in small cavities among the intestine contents (Pl. 10, fig. 1). No mycelium of *N. gossypii* was ever identified in the alimentary canal or living tissues.



Text-fig. 4. Partly diagrammatic median view of head of *Dysdercus* to show relative positions of feeding organs. Arrows indicate direction of sections shown in Text-figs. 5 A–D.

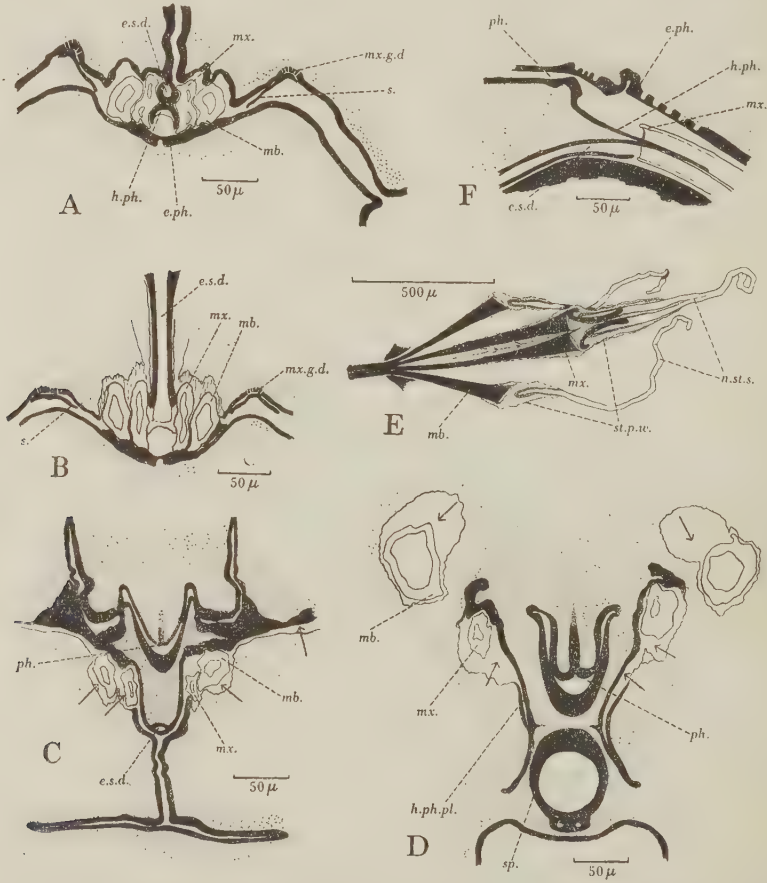
1st stage nymphs were able to suck up *Nematospora* spores; these could be subsequently detected either singly or in groups in the alimentary canal, sometimes in the oesophagus, but generally in the midgut. The spores are least easily observed at the anterior end of the midgut because the contents here become densely stained. Spores in the alimentary canal were detected up to 36 days* after the insect had been exposed to the fungus without a moult having

* Occasional insects are apparently unable to undergo the next moult, and remain at a certain stage of development for much longer than normal. This high figure of 36 days was obtained from a fourth nymph in this condition.

In addition to the spores of *Nematospora* in the intestine, others were accumulated near the bases of the stylets in the stylet pouches, behind the junction of pharynx and food channel. They were always retained by the chitinous lining of the stylet pouches, and never within the living tissues. These spores were easily recognized, singly or in large masses, and were often arranged parallel to the length of the stylets (Pl. 10, fig. 6). Unlike the spores in the intestine, those lodged in the stylet pouches were regularly observed to be germinating or to have produced a rather fine mycelium (Pl. 10, fig. 7). Twenty insects which were alive when fixed, representing all stages except 1st instars, had spores

actually germinating in the stylet pouches (Pl. 10, fig. 5), while thirty-five others had mycelium mingled with the spores. Fixing was carried out 1–36 days after exposure of the insects to *Nematospora*. Hence, spores can germinate within 24 hr. in the stylet pouches, presumably if they are mature before entering the insect. In twelve other insects, examined 1–9 days after feeding, only ungerminated spores were found in the stylet pouches.

Ullyett's observations (1930) that 1st stage nymphs remain underground and do not feed, were borne out here. The intestine contents formed a uniformly stained mass unlike that of any subsequent stage; no *Nematospora* spores were observed inside 1st stage nymphs after direct exposure to cultures for 2 days, although numerous spores adhered to the exterior of the insects.



Text-fig. 5. A. Section through head of 4th stage nymph showing junction of food channel with pharynx. B. Section through head of 5th stage nymph showing junction of salivary duct with salivary channel. C. Transverse section through head of 4th stage nymph posterior to junction of pharynx and food channel—positions where *Nematospora* spores accumulate indicated by arrows. D. Section further back than C, with each stylet in separate pouch—positions of *Nematospora* spores indicated by arrows. E. Basal parts of stylets in exuviae showing sheaths from new stylets, and chitinous lining of stylet pouches—positions of *Nematospora* spores indicated by arrows. F. Median longitudinal section of 5th stage nymph head at junction of pharynx and salivary duct with food and salivary channels (enlarged from Text-fig. 4).

Although the spores are pointed at both ends, no evidence was obtained that they, or the mycelium, could penetrate into the living tissues of the insect; in every case the fungus appeared to be confined to the lumen of the alimentary canal or to the space limited by the chitinous lining of the stylet pouches. Any penetration of the chitin would be the more

easily observed because the living tissues had contracted from it during fixing.

The figures show that spores occur in the stylet pouches at least as frequently as in the alimentary canal. Of insects where both head and intestine were completely examined, forty-nine had spores in both stylet pouches and intestine, seven in the stylet pouches only, and two in the intestine only.

In the stylet pouches, the *Nematospora* was associated with the bases of both maxillae and mandibles, although more frequently in contact with, or near the maxillae. Of forty-eight insects, thirty-three had *Nematospora* at the bases of both pairs of stylets, fourteen near the maxillae only, and one near the mandibles only. This is a contingency to be expected, since although the food channel is formed by the maxillae, these are closely interlocked with the mandibles until farther back where each is lodged in a separate pouch (Text-figs. 5 A, B, C, D).

(*d*) *Reisolation of the fungus*

To supplement the observation of germinating spores in the stylet pouches, confirmation that the fungus was present there in the living condition was sought by reisolation. Reisolation from the intestine was also attempted.

The method used was essentially similar to that of Caldis (1930) for isolation of yeasts from fig beetles. After insensitizing with chloroform vapour, insects were momentarily dipped in 90 % alcohol to remove surface air bubbles and then surface-sterilized by immersion in a 1:1000 mercuric chloride solution in 50 % alcohol for 3 min. Excess mercuric chloride was removed by using several changes of sterile water. With *Dysdercus*, the insect heads were then dissected and appropriate pieces crushed in potato-sucrose agar at 45° C. *Nematospora* was successfully reisolated from the heads of two 3rd stage nymphs previously exposed to a culture of the fungus. Difficulty was experienced because of the presence of other organisms more vigorous than *Nematospora*, particularly a white Gram-negative bacterium. In subsequent tests 0·05 % by weight of potassium tellurite (Conradi, H. & Troch, P. (1912), *Münch. med. Wschr.* LIX, 1652) dissolved in sterile water was added to the medium after autoclaving, as a bacterial inhibitor. By this method *N. gossypii* was reisolated from the basal regions of the stylets of a further three contaminated insects.

Attempts to isolate *Nematospora* from the intestines of the same insects were unsuccessful. This may have been due to the rapid development of other fungi, particularly *Fusarium*, *Eurotium*, *Rhizopus* and *Mucor*. Since, however, a slow-growing fungus, awaiting identification,* regularly appeared and was easily isolated in pure culture, the absence of *Nematospora* may have been due to the non-survival of the spores in the intestine.

The fungus mentioned above was regularly present in *Dysdercus* individuals, whether they had been previously exposed to *Nematospora* or not. A similar organism was described and isolated from the fat bodies of scale insects by Brues & Glaser (1921).

(*e*) *Effect of moulting*

Mechanics of the moulting process. The moult from 5th stage nymph to adult takes about 20 min.; in younger stages it is correspondingly shorter. Insects usually moult in a vertical position with the head down, an attitude probably assisting emergence from the skin by gravity. The skin first splits down the back of the thorax and the head becomes bent under the body with the antennae folded back parallel to the proboscis. The head, antennae and legs are next pulled out; finally, the abdomen is freed and the insect moves away. The fresh chitin takes some time to harden, and newly moulted insects are occasionally attacked by others which apparently puncture the abdomen and feed on the contents of the intestine. During the moult the old stylets are completely cast off, as are the salivary-pump casing and piston, the wall of the pharynx and the chitinous lining of the stylet pouches. The new stylets are previously formed as coils in the thorax, each within a chitinous sheath. The tip of the new stylet faces the base of the old one which it is about to replace. While the head is being pulled away from the old stylets during moulting, the new ones uncoil and are carried forward to the normal position. Finally, each new stylet is pulled out of its sheath and these sheaths remain on the exuviae forming four well-marked whitish strands behind the stylets (Text-fig. 5 E). The final stages in moulting, where the new stylets are being drawn out of their sheaths, can be watched under a low-power objective. Snodgrass (1927) describes similar sheaths round the new stylets in the *Cicada*. The central hollows in the stylet bases are in direct communication with the body cavity of the insect and contain living cells.

From the point of view of the transmission of *Nematospora*, it is important to ascertain whether there is continuity between these sheaths surrounding the new stylets and the chitinous lining of the stylet pouches. If such were the case, it might be possible for the new stylets to come in contact with the mass of spores in the stylet pouch, and thus the newly moulted insect would still be infective. Direct observation of sections, whole specimens cleared in caustic potash, and exuviae, indicate that this is improbable. The part of the sheath covering the tip of the new stylet is thickened and is present intact in exuviae, surrounded by the collapsed folds

* This fungus has been submitted to the Imperial Mycological Institute.

of the stylet pouch lining (Text-fig. 5 E). During the moult the tip of the new stylet breaks through the thinner part of the sheath laterally and the sheath is split up one side as the stylet is pulled out. Throughout, however, the old exuviae are pulled steadily farther away from the new stylets and it seems very unlikely that contamination from spores in the old stylet pouches could result.

Experimental observations. Insects at different stages of development were exposed to cultures of *Nematospora* and then allowed to pass the next moult before fixing. In the interests of clarity, the major conclusions derived from a considerable body of observation may first be summarized as follows:

(1) Spores are not retained in the stylet pouches after moulting; those retained in the intestine do not appear to remain viable.

(2) Recontamination occurs after moulting if a source of *Nematospora* is present in the environment, e.g. contaminated exuviae.

The details in support of these conclusions will now be presented.

A total of 104 insects varying from 2nd to 5th stage nymphs were exposed to *Nematospora* and allowed to moult before fixing; fifty-three showed no evidence of having fed on the fungus culture. Feeding tends to cease in nymphs as the next moult approaches; this, together with the fact that a number of the insects were fed through cellophane, may account for this high proportion which had not fed. Of the remainder (fifty-one in number), all had *Nematospora* in the stylet pouches of the exuviae (fourteen exuviae were not retrieved), 43 % had ungerminated spores in the hinder part of the intestine and 27 % spores, sometimes germinating, in the stylet pouches after the moult (see Appendix, Tables 2A, B). All of the insects with spores in the stylet pouches after moulting were 5th stage nymphs and had been left undisturbed in breeding dishes until 6–10 days after moulting from 4th to 5th stage; the spore mass in the pouches was mingled with a considerable proportion of bacteria. Since no spores were present here after moulting in the other insects which were immediately fixed or transferred to dishes free from *Nematospora* and exuviae, it is concluded that these insects had become recontaminated subsequent to the moult. Further support for this view resulted from a test experiment using insects fed through cellophane, so that spores could not be carried to the breeding dish on the surface of the body. In these, a week after moulting, only 18 % had spores in the stylet pouches compared with 86 % of those exposed direct to the *Nematospora* cultures and left undisturbed after moulting. The numbers of spores present in the stylet pouches after moulting were considerably less in the insects fed through cellophane.

No evidence was obtained to indicate that irregularities in the moulting process had occurred in any of these insects; no piece of the chitinous lining of the stylet pouch appeared to have been left behind.

The spores present in the intestines after moulting were here never observed to be germinating, while frequently the thin-walled ends were shrunken as though partly digested. Failure to isolate *Nematospora* from the intestines of recently fed insects, although spores were present, also indicates that spores may not remain viable in the intestine.

(*f*) *Survival of* Nematospora *spores*

Because of the indications that spores are not generally present in the stylet pouches after moulting, but that insects may subsequently become reinfected from random spores, perhaps associated with the exuviae, questions arise as to the viability of spores and the extent of their distribution in nature, apart from infected fruits and seeds.

Stainers are gregarious, the nymphs especially settling in groups on the stems and leaves of the cotton plants if not disturbed; accordingly, the accumulated exuviae constitute a potential source of spores for reinfection. Insect-contaminated extrafloral nectaries might also harbour the fungus.

(i) *Effect of humidity on spore survival.* Some viability experiments were carried out in which spores were subjected to atmospheres of known relative humidity and temperature. Constant humidities were maintained over saturated aqueous solutions of inorganic salts, only stable salts (mainly chlorides and sulphates) being used to preclude toxic effects which might result from decomposition. Humidity data were compiled mainly from *International Critical Tables.* Tests of the ability of the spores to germinate and grow were made after exposure to temperatures of 24 and 30° C. and relative humidities of 15–98 %; the results are indicated in Table 1.

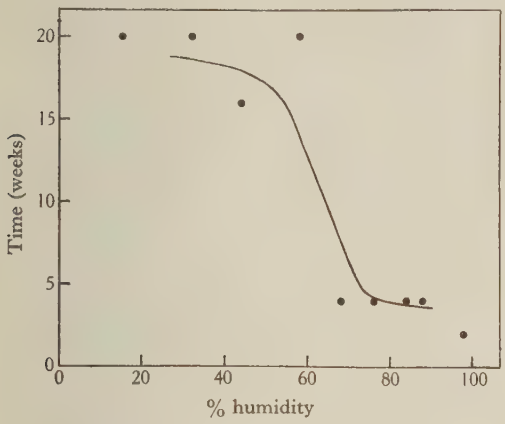
TABLE 1. *Effect of atmospheric humidity on survival of* Nematospora *spores*

Relative humidity* %	Time of survival of spores (weeks)	
	24° C.	30° C.
98	2†	—
88	4	1½
84	4	—
76	4	4
65	4	—
58	20	8
42	16	—
31	20	—
15	18	8

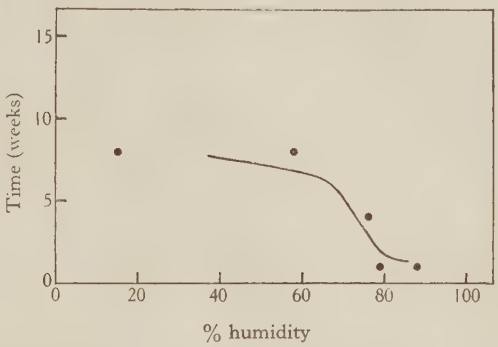
* Approx. values. † Contaminated after this.

These figures yield Graphs 1 and 2. It will be noted that there is a marked rise in spore survival below 70 % relative humidity at both temperatures.

In nature neither temperature nor relative humidity remains constant; there is normally a rise in temperature and fall in humidity by day and a reversal of this by night. Table 2 (from the *Reports received from Experiment Stations* of the Empire Cotton Growing Corporation) gives annual extremes of temperature and humidity recorded in some cotton growing areas since 1930. With rapid natural changes of humidity and temperature, *Nematospora* spores would probably remain viable for much shorter periods.



Graph I. Time of spore survival/% humidity at 24° C



Graph 2. Time of spore survival/% humidity at 30° C

TABLE 2. *Relative humidity and temperature data*

Locality	Year	Period when stainers most frequent	Annual extremes of humidity %	Annual extremes of temp. ° F.	Humidity extremes when stainers on cotton %	Temp. extremes when stainers on cotton ° F.
N. Rhodesia	1929–30	Mar.–July	53– 88	42– 97	53–88	42– 88
S. Rhodesia	1929–30	Feb.–May	32– 90	38– 97	53–89	43– 88
N. Rhodesia	1930–31	Mar.–July	50– 85	45– 96	50–79	45– 86
S. Rhodesia	1930–31	Feb. onwards	35– 98	39– 97	45–98	48– 92
Iraq	1930	No stainers	10–100	28–118	—	—
Iraq	1931	No stainers	15–100	22–115	—	—
N. Rhodesia	1931–32	Mar.–July	54– 92	45– 95	54–80	45– 81
S. Rhodesia	1931–32	Feb. onwards	30– 91	37– 96	48–91	48– 83
Iraq	1932	No stainers	10– 95	24–117	—	—
N. Rhodesia	1932–33	Mar.–July	23– 97	48– 98	45–90	48– 90
S. Rhodesia	1932–33	—	30– 94	42– 95	—	—
Queensland	1932–33	Dec.–Apr.	—	25–100	—	50–100
N. Rhodesia	1934–35	Mar.–July	36– 97	53–100	53–87	53– 86
S. Rhodesia	1934–35	Mar.–July	25– 93	35– 95	51–78	35– 87

(ii) *Inoculation of leaf nectaries.* For these experiments young plants were grown in pots until about 2 ft. high. A copious secretion of fluid from the leaf nectaries was noted. This was inoculated by needle with a piece of sporulating culture of *N. gossypii.* After a week it was observed that secretion tended to stop,* that there was no invasion of the leaf tissues by the fungus, but that *Nematospora* spores and mycelium were present and that occasional spores had germinated. Attempts at reisolation were unsuccessful. After 2 weeks similar results were obtained.

(g) *Infectivity of insects after exposure to* Nematospora

(i) *Using cotton bolls.* Eighty-six insects, ranging from 2nd stage nymphs to adults, have been tested for infectivity on cotton bolls. Of these, fifty-three successfully punctured the bolls, and twenty-six transmitted *Nematospora* to forty bolls. The insects were trapped in muslin bags measuring roughly 4 × 4 in. and tied over the selected bolls. The time that elapsed between the exposure of the insect to the

* Whether due to the fungus or the mechanical stimulus of inoculation was not ascertained.

fungus culture and bagging it on a cotton boll was varied; in some instances the insects were allowed to pass the next moult before testing. Some insects were fed on a succession of bolls, others on one only. In addition to transmission of *Nematospora*, a few cases of bacterial boll disease resulted. These are not included above.

Insect punctures, even when sterile, are easily detected after the cotton boll has been cut open, since in young bolls, the cells of the middle layer of the boll wall are stimulated to divide, and a mass of proliferated tissue is produced; this is forced inwards through the puncture hole and spreads out forming a cushion between the lint and the wall (Text-fig. 3 C). The dark centre usually present in the proliferation indicates where the proboscis had passed through (Pl. 10, fig. 4). In older bolls where active growth has ceased, a minute speck on the inner side of the wall indicates the location of the puncture. A slight yellow stain is present on the lint under a sterile puncture.

When *Nematospora* infection results, the yellow or brown staining of the lint is much more conspicuous although always confined to the loculus of the boll which has been punctured. No case of infection was recorded as being positive, however, unless *Nematospora* spores were actually observed in the diseased lint. Since 6 days' growth of *Nematospora* is required in the boll, before spores can be recognized, young bolls shed soon after puncturing, although possibly harbouring infections, were excluded.

The cotton boll is not equally susceptible to *Nematospora* attack throughout its development. In the later stages when vacuolation of the lint hairs has occurred, staining is much less severe than in bolls up to 4 weeks old (Pearson, 1934; Laycock, 1925). Tests for infectivity were accordingly carried out on young bolls. The average age of bolls punctured and infected by insects (18·5 days) was not found to be significantly different from that of bolls punctured by the same insects but not infected (20·3 days). (Appendix, Table 3.) The average age of bolls shed, presumably because of puncturing, was 6·6 days. Flowers were labelled on the second day of blooming and the ages of bolls which subsequently developed reckoned from that date.

The fifty-three insects which successfully punctured bolls were distributed as follows:

2nd stage nymphs	8 (3 infective)
3rd stage nymphs	10 (3 infective)
4th stage nymphs	15 (6 infective)
5th stage nymphs	9 (7 infective)
Adults	11 (7 infective)

They were subsequently fixed and sectioned.

The results will be considered in three groups (see Appendix, Tables 4 A, B, C).

(1) *Insects which punctured bolls and were examined before the next moult.* Twenty-eight insects belong to this group, including all the adults. Of these, nineteen caused infections.

(2) *Insects which punctured only after moulting.* Of seventeen here, two transmitted *Nematospora*. (See paragraph on moulting, p. 280.)

(3) *Insects which punctured before and after moulting.* Only eight insects successfully punctured both before and after moulting; five were infective before, and none after the moult.

The twenty-six infective insects (four were decayed and not sectioned) had all spores of *Nematospora* in the stylet pouches of the stage at which the insect was infective, except for one adult. In nineteen, mycelium was mingled with the spores and the fungus was in contact with the stylet bases. The intestines of fifteen were examined and ungerminated *Nematospora* spores observed in eleven. All stages of nymph and adult (except 1st stage nymphs, which apparently do not feed) were proved able to transmit the disease, although in nature, indications are that 2nd and 3rd stage nymphs are not important as vectors, mainly because they can only puncture exposed seeds or young bolls which do not provide adequate nourishment (Pearson, 1934; Wickens, 1938–9); the chances of their being contaminated are thus less.

There appeared to be no definite relationship between time elapsing after exposure to *Nematospora* and ability to infect (Table 3).

TABLE 3. *Time elapsing between exposure of each insect to* Nematospora *and subsequent cases of infection*

Stage when exposed to fungus	Time elapsing before each infection (days)
Adult	5, 7
,,	17, 55
,,	6
,,	1, 8, 9, 11
,,	4
,,	3
,,	2
5th nymph	1, 3, 5, 8, 10, 11
,,	1, 3, 5, 10, 11
,,	2
,,	1–8
4th nymph (infective as 5th)	5 days after moulting
4th nymph (infective as 5th)	,,
4th nymph	3
,,	1
,,	6
,,	2, 7
,,	1
3rd nymph	2, 6
,,	1, 3
,,	2
2nd nymph	1–7
,,	1–7
,,	1–7

Infection can occur 1–55 days after contamination: there is no characteristic time lag such as occurs when

a pathogen undergoes an obligatory part of its life history inside its insect vector.

(ii) *Using sterile culture media.* The idea was entertained that by allowing infective insects to puncture through cellophane superimposed on a thin layer of culture medium, it might be possible to ascertain the nature or condition of the fungus on being discharged from the insect. Accordingly, insects were trapped inside tubes covered at one end by sterilized cellophane, and the cellophane end then laid on a slide covered with a thin layer of sterilized cotton seed or potato-sucrose agar, the whole being contained in a sterile jar, i.e. the only part not sterile was the cavity containing the insect.

Of thirty-nine insects tested in this way, varying from 2nd stage nymphs to adults, twenty-six had apparently penetrated the cellophane, but only in one case did an infection of *Nematospora* result. Almost invariably, a variety of contaminants was carried through to the agar, including the fungus described on p. 279, and frequent bacteria, yeasts and moulds. It was not possible to observe whether the one resulting *Nematospora* colony originated from a spore or not.

Subsequent microscopic examination revealed that 30 % of these insects had *Nematospora* spores in the stylet pouches. The higher proportion of cotton bolls to agar plates infected by contaminated insects may be due to the selective action of the living host plant in eliminating saprophytes.

(*h*) *Observations on other species of fungus and insect*

Dysdercus Howardi. A few individuals of *D. Howardi* (kindly supplied by Miss E. I. MacGill from her stock) were fed on *Nematospora gossypii* cultures and subsequently examined. Of five insects (four males and one 5th stage nymph), one conveyed *Nematospora* to two cotton bolls, three had spores in the stylet pouches, and four in the intestine. These observations indicate that the same relationship exists with *Dysdercus Howardi.*

Nematospora coryli. Individuals of *Dysdercus intermedius* of all stages were exposed direct to cultures of *Nematospora coryli* on cotton-seed kernels. Subsequent examination of these insects proved that this fungus is also taken up and becomes lodged either in the stylet pouches or alimentary canal. Ungerminated spores (Pl. 10, fig. 3) and also possible fragments of mycelium were present in the alimentary canal, while in the stylet pouches spores had germinated and mycelial buds were present. The proportion of spores to buds in the stylet pouches was higher than in the original culture. *N. coryli* was successfully reisolated from the heads of two 4th stage nymphs, using the potassium tellurite technique already described (see p. 279).

(*i*) *Possibility of change in morphology of fungus*

Some parasites alter in morphology when living in their host vector. The possibility of such changes in *Nematospora* was explored by the following methods:

(*a*) *Effect of inoculation into cotton-bolls*

N. gossypii after reisolation from inoculated cotton bolls was entirely unchanged in morphology; there was a slight increase in growth rate. (Detailed figures in Appendix, Table 5.)

(*b*) *Effect of insect extracts on the fungus*

In the first experiments of this type, an aqueous extract of freshly killed insects was added to the culture medium before sterilizing. No difference in fungus morphology resulted from use of this medium, although a slight increase in growth rate was recorded.

In subsequent tests, the insect extract was sterilized by use of a Seitz filter to avoid decomposition. Here, again, there was no change in the fungus morphology.

(*c*) *Regular bud formation in* Nematospora gossypii

Small buds frequently occur on the mycelium of *N. gossypii*, especially in the lower layers of old cultures (Text-fig. 1 C) or on plain agar (Text-fig. 1 D). Similar structures are figured by Ashby & Nowell (1926), who observed their formation direct from spores and their germination to give mycelium. Buds were also present in the cultures grown in presence of insect extracts but not more frequently than in the controls.

V. EXAMINATION OF MATERIAL FROM THE FIELD

Through the sending of fixed material of *Dysdercus* from Nyasaland by Dr E. O. Pearson, opportunity has arisen for comparison of these observations on insects bred in captivity with insects from natural sources. This field material comprised:

(*a*) Adult insects of both sexes of *D. intermedius* and *D. fasciatus* and fully-fed 5th stage nymphs of *D. fasciatus.* These insects had been fixed immediately after collection from a cotton field showing heavy stainer damage.

(*b*) Adult insects of *D. fasciatus* which had been collected from the field as 5th stage nymphs, and fixed immediately after moulting to adults in captivity.

Results obtained from examination of sections of these insects (Table 4) show that 60 % of the insects fixed immediately on collection from the field had *Nematospora* spores present in the stylet pouches. These spores were frequently mingled with mycelium and in some cases were actually germinating. *Nematospora* spores were present in the intestine in 63 % of these insects, but here there was no evidence of germination; frequently the spores in the intestine showed signs of disintegration.

None of the insects fixed immediately after moulting had *Nematospora* spores in the stylet pouches, although 71 % of these had ungerminated spores in the intestine.

There was no evidence of *Nematospora* in the living tissues of any of the insects. The body cavity of one insect, however, was parasitized by numerous crithidial flagellates.

These observations yield evidence to confirm that, in nature, as under artificial conditions, live *Nematospora* spores are harboured in the insect stylet pouches, but that they are cast off during moulting so that insects must become reinfected after each moult.

TABLE 4. *Examination of insects from the field*

Type of insect	No. examined	No. with *Nematospora* in stylet pouch	No. with *Nematospora* in intestine
D. fasciatus, female	7	5	4
D. fasciatus, male	7	4	4
D. fasciatus, 5th nymph	7	7	6
D. intermedius, female	4	2	4
D. intermedius, male	8	2	3
D. fasciatus, adults freshly moulted	21	—	15

VI. Discussion

The observation of *Nematospora* spores in the intestine and stylet pouches of *Dysdercus* spp. after exposure to infected cotton bolls or to cultures of the fungus, verifies that the insect is a true vector of internal boll disease.

Uptake of Nematospora

It is easy to understand how spores sucked up along with food material could reach the alimentary canal of the insect via the normal food channels. Their presence in the stylet pouches, however, is less easy to understand. Three alternative explanations are possible:

(1) There is a leakage during feeding. This can be supported by direct observation of the insect anatomy. Tower (1914) gives details of the junction between the food and salivary channels, with the pharynx and exit duct from the salivary pump respectively, in the squash bug. The ventral plate of the pharynx is prolonged forward forming the hypopharynx, which enters and lies along the ventral side of the food channel; the anterior end of the dorsal plate of the pharynx, the epipharynx, lies above the maxillae closing the top of the food channel where the stylets diverge. In *Dysdercus* a similar arrangement is found (Text-figs. 5A, F). There would thus be a gap on either side between epipharynx and stylets if all the parts were not tightly pressed together; through this, spores could pass into the stylet pouches. The exit duct from the salivary pump is tubular and lies completely inside the salivary channel (Text-fig. 5B). During the pumping-sucking process, these junctions must all be tightly pressed together or no suction would be possible; Tower assigned the function of keeping the junctions tight to the thin membrane investing the stylets in this region. In *Dysdercus*, the secretion from the maxillary glands apparently accumulates here (Text-figs. 5A, B) and may serve to seal and lubricate the junction. At the start and finish of the feeding process, however, it is necessary for the stylets to be moved backwards and forwards so that they bore through the surface of the cotton boll or seed; at these times there must be some relaxing of pressure round the stylets to enable them to be moved. During movement of the stylets, too, the action of the muscles attached to the chitinous wall of the stylet pouch, and also variations, even if slight, in the length of stylet within the pouch, will alter the volume of the stylet pouch. These cavities are likely to be filled with liquid, since evaporation from narrow openings is slow; any increase in volume would result in a suction carrying more fluid into the pouch. The maximum distance to which spores have been observed to penetrate in the stylet pouch beyond the junction region is about 1·5 mm. *Nematospora* spores are eminently suited to move with a fluid because of their pointed ends and large surface area in proportion to bulk. Some observations on the movement of *Nematospora* spore suspensions in capillary tubes did show that spores and also mycelial fragments were carried up in the column of liquid and accumulated under the meniscus. In the narrow insect food channel (maximum diameter 20μ) the spores would of necessity be arranged longitudinally, a position more suited for the pointed end to pass through any narrow opening. The spores of all the internal boll-disease fungi have pointed ends.

(2) The spores might enter via openings between the chitinous plates of the head. This has been disproved by the observation of spores in the stylet pouches of insects fed through cellophane (here only the ends of the stylets are in contact with the fungus). The percentage of insects with *Nematospora* in the stylet pouches after direct exposure to the fungus (70 %) is considerably greater than that for insects fed through cellophane (26 %), but this

is believed to be due to the mechanical difficulty of puncturing the cellophane.

(3) The spores might pass through the living tissue from the intestine and re-emerge. No evidence either of fungal spores in the body cavity or of their ability to penetrate the intestine wall or chitinous lining of the stylet pouches has been obtained. There is evidence that the spores do not remain viable in the intestine, since attempts at reisolation were unsuccessful, germinating spores in the intestine were not observed, and spores with the thin-walled end shrivelled were present.

Insects are infective immediately after feeding, without a time lag such as would occur if spores were moving through the body from the intestine.

Ejection of the fungus

The question of how the fungus leaves the insect must now be considered. Here again a number of possibilities arise:

(1) The fungus comes out by the same route as it has entered. Any decrease in the volume of the stylet pouch would result in the expulsion of liquid likely to contain spores if the insect is contaminated. It is possible also that mechanical shuffling of the spores along the stylet bases takes place while these are moving backwards and forwards during the puncturing process. Any spores reaching the top of the salivary channel would then be carried in the saliva on to the food supply. Suction from the pharynx might also help to draw materials out of the stylet pouches if there were any obstruction at the foot of the food channel during feeding.

(2) The salivary glands might become contaminated. Examination of the salivary glands, ducts and pump of contaminated insects did not reveal the presence of *Nematospora* or other organisms. To reach the salivary glands the fungus would invade living tissues, there would be a time lag before an insect could cause infections, and infections would thereafter occur with some regularity. This was not found to be the case.

(3) Regurgitation might occur from the intestine. Insects with a specialized salivary mechanism are generally believed not to regurgitate. In *Dysdercus*, the valve at the entrance to the midgut and the absence of evidence that spores remain viable in the intestine, indicate that regurgitation is an improbable explanation. In recent work on the transmission of typhus by the louse, where the pathogen is present in the gut and excreta of the insect, infection is believed to be by rubbing in of excreta, and not by insect regurgitation (Mackenzie, 1942).

(4) Infection could occur from excreta. The observations of Hansford (1929*a*) and others that the disease only develops when the fungus is introduced through the wall of the cotton boll indicates that the inoculum must be conveyed via the proboscis, and rules out the possibility of passive infection from excreta, should any viable spores still be present.

The efforts to observe the form in which the fungus leaves the insect were not successful, as no efficient method was found of making such observations. The spore is best suited, in shape and chances of survival, but pieces of mycelium or, especially in *Nematospora coryli*, vegetative buds might also be ejected.

Infectivity

In nature, the number of spore-carrying insects will vary with the incidence of the disease in any particular season. Pearson (1934) states that the stainer population tends to become saturated with the disease as the season advances. In the present investigation 61 % of the insects which punctured bolls were infective, excluding those which had previously moulted. The observations show that infections occur entirely at random, and support the view that ejection of the fungus is by chance.

Moulting

Although a high proportion of experimental insects (27 %) had spores in the stylet pouches after moulting, these were all bred under conditions where recontamination after the moult was possible. No spores were found in the stylet pouches of insects fixed immediately after moulting or where all sources of recontamination were removed.

Spores are retained in the intestine after moulting, but there is no evidence that they remain viable. Insects with spores present in the intestine but not in the stylet pouches after a moult did not prove to be infective.

In nature, the chief source of recontamination will be from infected fruits and seeds of cotton or alternative host plants, but exuviae and perhaps also extra-floral nectaries could harbour a possible inoculum of *Nematospora*. It has been shown that the spores are fairly resistant to conditions of constant low humidity and survive for about a fortnight in all humidities. Viable spores might also be present in diseased seed at ginneries or packing stations.

Similar fungi as arthropod parasites

Metschnikoff (1884) describes the fungus *Monospora bicuspidata*, which resembles *Nematospora coryli* in structure, forming large numbers of conidia and buds, some of which increase in size, become asci and produce a single needle-shaped spore. This fungus was parasitic in *Daphnia*, the water flea. Spores were swallowed which partly penetrated the gut wall and germinated to produce buds in the body cavity. These multiplied until the fungus practically filled the body cavity with buds, and asci containing spores. The presence of the fungus killed infected individuals in about 2 weeks. Metschnikoff (1884) was unsuccessful in isolating the *Monospora*.

Keilin (1920) records a similar infection of a dipterous larva by a fungus which he named *Monosporella unicuspidata*.

These are cases of true parasitism, unlike the passive relationship between *Nematospora* and *Dysdercus* where the insect is apparently unharmed, and there is no evidence that the fungus can enter the living tissue of the insect. No plant hosts have been recorded for *Monospora* or *Monosporella*.

Field material

Examination of insects from natural sources confirmed the experimental observations, and yielded no evidence to suggest that the fungus can enter the living tissues of the insect.

Wider implications

In the present work the writer has given detailed accounts of a specific instance of a plant disease in which the pathogen is definitely transmitted by an insect vector and solely by that means. This type of fungal disease is of relatively infrequent occurrence. It may therefore be of interest to inquire what value a specific investigation of this type has in the general framework of biology. The subject-matter falls on the border-line between entomology and mycology —a position which it shares with many other diseases. Some impression of the importance of these diseases may be obtained from a consideration of the recent comprehensive volume by Leach (1940) in which he shows how wide is the field for investigation in terms of the insect vectors and the fungal, bacterial and virus plant diseases which they disseminate; seventy virus, fourteen bacterial and twenty-five fungal diseases transmitted by 200 arthropod vectors (mainly insects) are listed as the more important examples. He indicates also how these instances of insect transmission can be classified according to the obligatory or mechanical relationship between pathogen and vector, if these facts are known.

Because of the medical implications, advances in our knowledge of the ways in which animal pathogens are transmitted by insect vectors have been more rapid and sustained than is the case with plant diseases, but it is obvious that these fields have much in common and that experimental techniques developed will in many cases be applicable to both. The use of plant hosts may often prove more suitable for the investigation of such techniques since they can be reared and handled with greater ease. Ecological aspects might also yield more readily to investigation when only one of the three organisms involved is actively mobile (the insect).

The subject presents interest also from the view points of symbiosis and of evolution. Leach points out the striking similarities between the role of insects in pollinating higher plants and in disseminating certain pathogens. In many cases also a balance has been set up between the insect and the pathogen (e.g. *Ceratostomella* sp. and bark beetles), while such associations have become vital for the survival of some organisms, an observation particularly applicable to the *Nematospora* fungi where the insect is the only means of dissemination, and to many plant viruses.

In conclusion, the investigation of such problems is of wide economic importance, leading as it often does to the application of suitable control measures with reasonable chances of success in checking or eliminating the pathogen.

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APPENDIX

TABLE 1. *Insects examined before moulting, after exposure to* Nematospora gossypii

A. Insects exposed direct to culture or spore suspension

Description of insect	No. examined	No. with spores in stylet pouch	No. with spores in intestine
Adult	12	8	4 (6 not examined)
5th nymph	8	7	4 (3 not examined)
4th nymph	16	15	12 (2 not examined)
3rd nymph	6	4	4
2nd nymph	11	6	5
1st nymph	6	—	—
Totals	59	40	29 (11 not examined)

Percentage with spores in stylet pouch = 75 %
Percentage with spores in intestine = 69 % } excluding 1st stage nymphs

B. Insects fed through cellophane

Description of insect	No. examined	No. with spores in stylet pouch	No. with spores in intestine
Adult	3	2	1
5th nymph	22	6	6
4th nymph	7	3	3
3rd nymph	12	4	2
2nd nymph	21	2	2
Totals	65	17	14

Percentage with spores in stylet pouch = 26 %
Percentage with spores in intestine = 21·5 %

C. Insects fed on inoculated cotton bolls

Description of insect	No. examined	No. with spores in stylet pouch	No. with spores in intestine
Adult	1	—	—
5th nymph	3	2	1

TABLE 2. *Effect of moulting*

A. Insects fed direct on culture

Stage when exposed to *Nematospora*	No. examined	Spores in stylet pouches of exuviae	Spores in stylet pouches after moult	Spores in intestine after moult
2nd nymph	1	1	—	—
3rd nymph	2	1 (1 not examined)	—	2
4th nymph	21	9 (12 not examined)	12	11 (1 not examined)
5th nymph	10	9 (1 not examined)		2 (2 not examined)
Totals	34	20 (14 not examined)	12	15 (3 not examined)

B. Insects fed through cellophane

Stage when exposed to *Nematospora*	No. examined	Spores in stylet pouches of exuviae	Spores in stylet pouches after moult	Spores in intestine after moult
2nd nymph	1	1	—	—
3rd nymph	5	5	—	1
4th nymph	10	10	2	6
5th nymph	1	1	—	—
Totals	17	17	2	7

TABLE 3. *Boll-infection by* Dysdercus. *Comparison between age, when punctured, of contaminated bolls and those which did not develop infection*

Age of infected bolls when punctured days	Age of bolls not infected when punctured by infective insects days	Age of infected bolls when punctured days	Age of bolls not infected when punctured by infective insects days
10	3	12	9
8	8	41	15
9	14	7	21
10	17	6	20
17	21	21	12
12	28	17	9
14	46	10	62
10	17	32	10
15	13		15
22	17		13
21	4		21
31	33		34
26	10		26
31	14		51
26	51		17
23	11		24
24	7		55
21	10		11
26	32		33
20	6		5
14	7		

TABLE 4. *Infectivity of insects exposed to* Nematospora

A. Insects which punctured only before moulting. Total 28

Stage	No.	No. infective	Spores in stylet pouches	Spores in intestine
2nd nymph	7	3	5	4
3rd nymph	3	3	3	3
4th nymph	6	5	6	4 (2 not examined)
5th nymph	1	1	Not examined	Not examined
Adult	11	7	6 (1 not examined)	5 (5 not examined)

B. Insects which punctured after moulting. Total 17

Stage when fed	Stage when puncture made	No.	No. infective	Spores in stylet pouches of exuviae	Spores in stylet pouches after moult	Spores in intestine
2nd nymph	3rd	1	—	—	—	—
3rd nymph	4th	5	—	— (3 not examined)	—	1
4th nymph	5th	7	2	2 (3 not examined)	2	4
5th nymph	Adult	4	—	3	—	—

C. Insects which punctured before and after moulting. Total 8

Stage when fed	No.	No. infective before moult	No. infective after moult	Spores in stylet pouches of exuviae	Spores in stylet pouches after moult	Spores in intestine
2nd nymph	—	—	—	—	—	—
3rd nymph	2	—	—	1	—	1
4th nymph	2	1	—	2	—	1
5th nymph	4	4	—	2 (2 not examined)	— (1 not examined)	1 (1 not examined)

TABLE 5. *Growth of* Nematospora. *Increased growth rate of* Nematospora *after inoculation into cotton bolls*

Source of fungus	Av. diam. of colony on potato-sucrose agar (mm.)				
	3 days	5 days	7 days	10 days	15 days
Original culture	25·5	34·5	46	63·5	70·5
1st reisolation	28·5	37	47·8	68	79
2nd reisolation	25	35·5	47·5	68	77
3rd reisolation	27·5	37	50	69	77
4th resiolation	27·2	37·8	50·5	70	76·8
∴ Av. for 4 reisolations	27	36·8	48·9	68·8	77·6

ADDENDUM

Since going to press, a further relevant paper has been noted (J. G. Leach & G. Clulo, 'Association between *Nematospora phaseoli* and the green stinkbug', *Phytopathology*, **33**, 1943, 1209–11). These authors consider that transmission is here also mechanical. They were able to isolate the fungus from the surface of insects from infected plants but not from the internal organs. While concluding that the vegetative cells of the fungus are too large to pass along the food or salivary channels of adult insects, the role of the spores in this connexion is not mentioned.

EXPLANATION OF PLATE 10

Fig. 1. Ungerminated spores of *N. gossypii* in intestine of 4th stage nymph fixed 6 days after exposure to a culture. × 1000.

Fig. 2. The only *Nematospora* spore in an insect intestine showing germination—from a 2nd stage nymph fixed immediately after exposure to a culture. × 1000.

Fig. 3. Ungerminated spore of *N. coryli* in intestine of 5th stage nymph. × 1000.

Fig. 4. Transverse section of cotton-boll wall showing puncture by 2nd stage nymph. × 75.

Fig. 5. Germinating spore of *N. gossypii* in maxillary stylet-pouch of 5th stage nymph. Other spores and mycelium also present. × 1000.

Fig. 6. Spores of *N. gossypii* in stylet pouch of 4th stage nymph—some spores in contact with maxilla. × 1000.

Fig. 7. Spores and mycelium of *N. gossypii* within chitinous lining of maxillary stylet pouch in 4th stage nymph. × 500.

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Dry rot of potato [*Fusarium caeruleum* (Lib.) Sacc.]. Investigation on the sources and time of infection

By T. Small, Ph.D., A.R.C.S., *Agricultural Advisory Department, Manchester University*

Inoculation with soil samples proved that the fungus causing dry rot is frequently present in field soils in Cheshire and in soil adhering to imported seed tubers. The fungus was viable in soils having a wide range of *p*H values and in fields which had not grown potatoes for 5–6 years. Other sources of infection include lofts, used sacks, seed boxes, diseased tubers, and knives used for cutting seed potatoes.

Bruised tubers stored in heavily contaminated boxes developed much dry rot; far less disease occurred in unbruised tubers. In boxes containing own-saved seed, healthy tubers in contact with diseased ones remained sound. Bruised tubers in contact with, or contaminated by, diseased tubers contracted dry rot. Cutting seed with a contaminated knife increased the disease seven-fold.

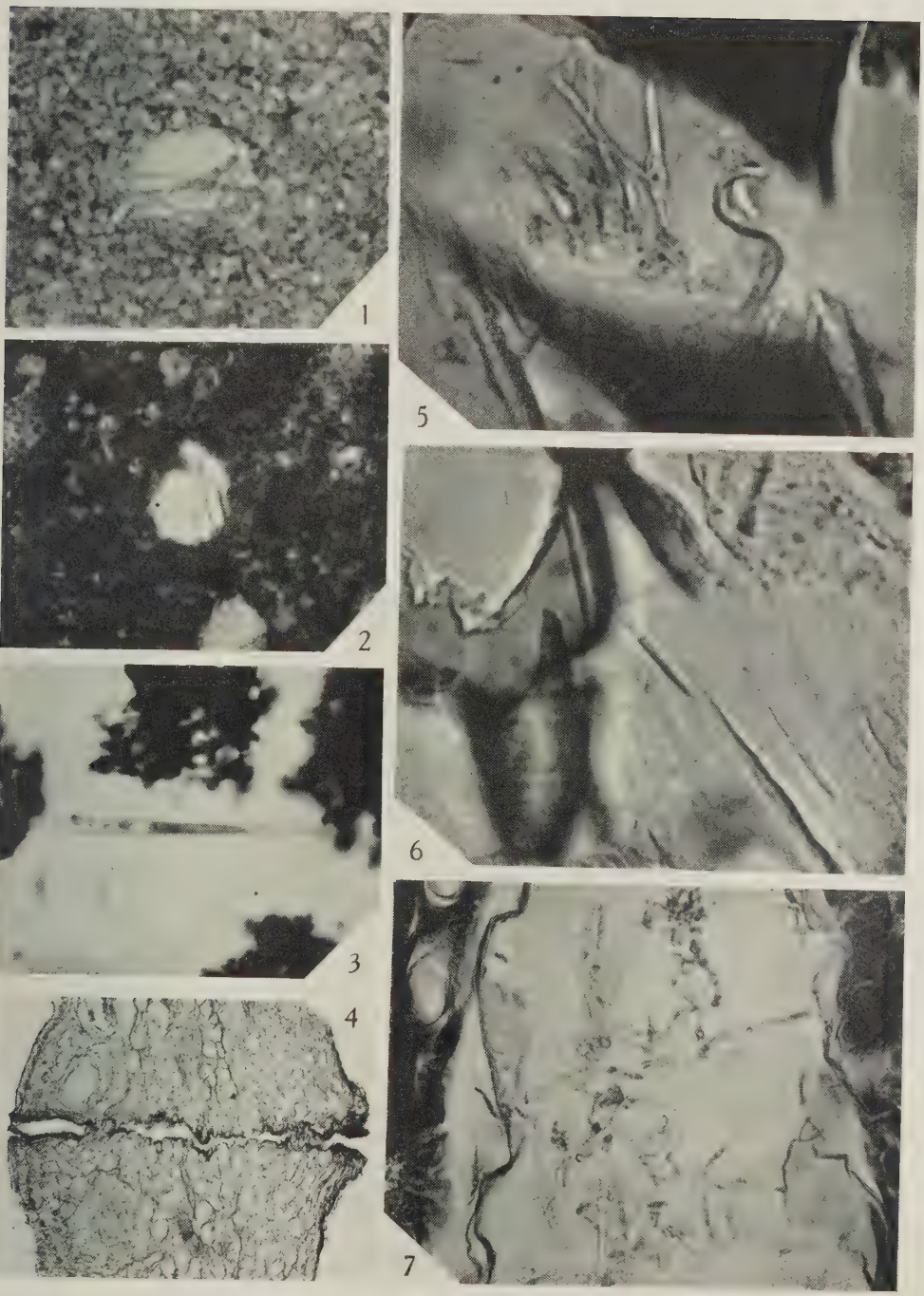
Inoculation of tubers attached to the parent plant showed that little or no infection occurred before lifting. In field trials severe dry rot developed in several varieties 6–8 weeks after lifting.

The results are discussed in relation to seed treatment.

Since Pethybridge & Lafferty (1917) investigated dry rot in Ireland little experimental work has been published in the British Isles. Foister (1940) wrote a general account of the disease in Scotland, and Foister & Wilson (1943) published a summary of some recent experiments. A study of the literature suggested that an investigation was needed on the sources and time of infection; an account of this work is given here.

Experimental

Pure cultures, spore suspensions and inoculations. At the beginning of this work single-spore cultures of *Fusarium caeruleum* were made; to avoid staling, the fungus was inoculated into potato tubers and reisolated at approximately 6-month intervals. Spore suspensions were made from pure cultures in tap water to which a little raw tuber was added and



Figs. 1–7

were always tested in hanging drops and by inoculation into susceptible tubers. Internal inoculations were made, as described by Pethybridge & Lafferty (1917), by removing a cone-shaped piece of tissue (leaving the skin intact at one place to act as a hinge), placing the inoculum in contact with the flesh, and replacing the cone. Spore suspensions were atomized over the outside of the tubers.

Variety and wounding of tubers. Var. Ninetyfold was used unless otherwise stated, because it is very susceptible and is rarely attacked by blight (*Phytophthora infestans*) in this area: Doon Star, another susceptible variety, was used in certain tests. The term 'mature' is used to describe tubers taken from store from December onwards. The 'standard bruise' consisted of shaking the sterilized hamper containing the tubers 30 times; a more severe bruise was usually obtained by standing on, and rough handling of, sacks containing tubers. In laboratory tests the tough skin of mature tubers was removed in several places with a file.

Controls and storage. Dry rot developed occasionally in non-inoculated control tubers but was seldom sufficient to invalidate the results. Unwounded tubers were carefully selected, but some may have carried small wounds through which infection occurred. The boxes used for storage in almost all the field trials were ordinary seed boxes.

Sources of infection

Soil

It has long been suspected that the fungus causing dry rot lives in the soil, but, so far as the writer is aware, no proof, based on experimental work, has been published. Pethybridge & Lafferty (1917) concluded that presumably the fungus is a normal inhabitant of the soil, while Foister (1940) states that 'it has been proved that the organism causing dry rot can live in the soil and infect tubers while these are still in the soil', but no experimental details are given.

Inoculation with soil. In these tests (Small, 1944) tubers were inoculated internally with soil scraped from tubers with a sterile knife and collected in new envelopes; eighteen inoculations were made with each soil sample. In earlier trials one-half of each sample was used dry, the other half after being mixed with sterile water; the results were similar and so the samples were used in a dry state in later work. Controls were inoculated with pure culture (to prove that the test tubers were susceptible) or were cut but not inoculated.

The samples (each was collected from thirty tubers) were taken, (*a*) on twelve Cheshire farms in the field at digging time, before the tubers came into contact with any source of infection such as hampers, boxes or sacks; (*b*) from the farmer's own-saved seed (stored in boxes in lofts) 2–3 weeks after lifting in July and before any dry rot appeared; (*c*) from healthy seed in boxes during the winter; (*d*) from sacks of seed imported from Scotland and Northern Ireland, stored in merchants' warehouses, before being distributed to growers; from each consignment ten of the topmost tubers were taken from each of three sacks—sacks seen to contain diseased tubers (there were few of these) were not sampled.

The results, summarized in Table 1, show that almost every soil sample was contaminated; those collected in the field prove that the field soil was infected and indicate that the fungus is widely distributed in Cheshire soils; in four cases potatoes had not been grown in the soil for 5–6 years and in one case for at least 9 years. The rest of the samples may have been contaminated in the lofts, boxes or sacks, or by soil falling from one consignment to another in transit, but the results show that soil on healthy seed stored in boxes or imported from Scotland and Northern Ireland is frequently contaminated. The fungus was viable in soils having *p*H values between 5·25 and 6·74. It follows from these results that soil on loft floors and in used sacks is often contaminated; when lofts are swept the contaminated soil particles afterwards settle on the seed and boxes.

Table 1. *Inoculation with soil samples*

Source of soil samples	No. samples	Contaminated soil samples	
		No.	Range of *p*H values
Tubers in field at digging time	18	18	5·26–5·68
Tubers in boxes 2–3 weeks after lifting	16	15	5·25–5·85
Tubers in boxes during winter	27	25	— —
Imported seed	42	37	5·50–6·74

During this work reisolations and reinoculations were made frequently; the reisolated cultures have not yet been identified except as *Fusarium* sp. which caused dry rot when inoculated into tubers. It may be noted here that unbruised seed saved from the above infected Cheshire fields, suffered little loss except at one farm.

Diseased tubers

Spread of dry rot from diseased to healthy tubers in boxes. In laboratory tests mature, wounded (file) and unwounded tubers were placed in contact with diseased tubers bearing numerous spore pustules and were stored in moist and dry chambers. In both

chambers almost all the wounded but few of the unwounded contracted dry rot.

Trials were made on eight farms in 1942 and again in 1943, using the farmer's own-saved seed. The tubers were lifted with a 'spinner' in July and at digging time were placed in boxes or were 'standard' bruised and then placed in boxes; one-half of each series (bruised and unbruised) was dipped for 1 min. in a proprietary organo-mercury preparation. Dry rot appeared in September, and in October the affected tubers (few in the unbruised, undipped, but numerous in the bruised, undipped) were discarded, and forty selected diseased tubers bearing spore pustules were placed in contact with the healthy tubers left in each box except the controls. In February the increase in dry rot in the dipped, undipped, and control boxes was negligible. Farmers are often advised to examine their seed potatoes regularly and to discard diseased tubers to prevent the spread of infection; this is laborious and costly and the above results suggest that it is unnecessary where, as is usual in Lancashire and Cheshire, growers lift and store their own-grown seed in boxes.

The results also showed that Ninetyfold, dug and bruised in July may develop serious dry rot (20–50 %) as early as October, and that practically no further loss occurred after late October.

Diseased tubers in clamps and in sacks. In laboratory tests washed, mature, wounded (file) tubers of Ninetyfold, Majestic and Doon Star were placed in sterile sacks with a few diseased tubers bearing spore pustules, shaken several times and then stored in moist chambers. Much dry rot developed, thus showing that the infection had spread from the diseased tubers. Controls remained sound. The extent of this contamination during grading, boxing and other processes has not been ascertained in practice, but it is possibly important only where the soil on the tubers is not already contaminated.

Cutting seed tubers. This practice is known to spread dry rot, but the extent of the increase has not been investigated. In these tests one diseased tuber was cut and then the knife was used to cut eight mature seed consecutively; six tests were made and the ninety-six half-tubers were stored in a moist chamber. Controls, cut with a sterilized knife, remained healthy. Forty-two halves developed dry rot, a seven-fold increase; in the six tests the disease was spread to the 4th, 5th, 7th, 7th, 7th and 8th tubers respectively. Treatment of the half-tubers with an organo-mercury preparation (used as directed) immediately after cutting, failed to check the disease sufficiently.

Contaminated knives may cause serious loss when cutting is done several weeks before planting; the writer has inspected such cases. Where, however, the cutting is delayed until planting time, the result depends upon the rate of disease development and plant establishment, a point that can be settled only by field trials; these are now in progress.

Seed boxes

Clean, mature, wounded (file) and unwounded tubers were placed in contaminated boxes (sprayed with a spore suspension and allowed to dry) and stored in moist and dry chambers. In both chambers much dry rot developed on the wounded tubers but little in the unwounded.

The test was continued under farm conditions. Diseased tubers bearing spore pustules were stored for several weeks in boxes; the boxes were emptied and refilled at once with healthy tubers, which were gently rolled to ensure contact with the box. Some tubers were washed, dried, and bruised (in a sterile sack), before being placed in the boxes. Controls were kept in new boxes. The treatment and results of one trial are given in Table 2; losses under 1 % are entered as nil. The Ninetyfold were dug in July and used at once, whilst the Majestic were taken from store in December; each series included three boxes, each containing 100 tubers.

TABLE 2. *Development of dry rot in tubers stored in contaminated boxes*

Variety and treatment of tubers	Contaminated boxes; dry rot (%)	New boxes; dry rot (%)
Ninetyfold:		
Not washed, not bruised	1	0
Washed, bruised	13	0
Washed, inoculated, bruised*	—	39
Majestic:		
Not washed, not bruised	9	1
Washed, bruised	56	4

* This series, inoculated with a spore suspension, was included to test if the freshly dug Ninetyfold were susceptible.

The results show that contaminated boxes may be a source of infection especially when the tubers are bruised; relatively little loss occurred in the unbruised tubers, although the boxes were far more heavily contaminated than would usually occur in practice. Where the soil on the tubers is contaminated, contaminated boxes are likely to be unimportant; where the soil is not contaminated, it is doubtful if such boxes cause extensive damage except perhaps in rare cases where severely bruised tubers are placed in heavily contaminated boxes. Treatment of the boxes may be worth while only after severe outbreaks.

Incidentally the small loss in the washed, bruised tubers in the new boxes suggests that little, if any, infection occurs before lifting. The bruising of washed and unwashed tubers has also been used to

show that the soil is a source of infection; in one test with approximately 500 freshly dug tubers removal of the soil by washing reduced dry rot from 20·7 to 3·1 % and in another from 10·4 % to nil.

TIME OF INFECTION

It has been shown that field soil and soil adhering to seed tubers after lifting is frequently contaminated. Efforts were made to ascertain when infection occurs.

Preliminary tests. Two unsterilized field soils (*p*H 5·5 and 6·8) were inoculated with a spore suspension, and were made to adhere to surface disinfected, mature, wounded (file) and unwounded tubers, which were then stored in moist chambers. Infection occurred in the wounded and unwounded tubers, whilst the controls (treated with sterilized soil) remained sound. In other trials mature, wounded (file) and unwounded tubers, inoculated with a spore suspension and allowed to dry, were buried in moist or dry soil in closed boxes; in one test the soil-moisture content, expressed as percentages of the wet samples, was 15·5 and 5·8 and in another it was 18·4 and 6·7; the *p*H values were 6·6 and 5·4 respectively. Much dry rot occurred in the wounded tubers and a little in the unwounded. Similar results were obtained when mature, inoculated (with a spore suspension) tubers were buried in soil (*p*H 5·1) in the open in November and June.

The results show that mature, wounded and unwounded, tubers, contracted dry rot when placed in contact with contaminated soil and that infection occurred when mature inoculated tubers were buried in soils having a wide range of temperature, moisture and *p*H values.

Inoculation of growing tubers

Pot trial. One seed tuber was planted 13 May in each of forty 10 in. pots filled with field soil (*p*H 6·7). Ten pots (controls) remained uninoculated; the remainder were inoculated by spraying 100 c.c. of a spore suspension over each seed tuber and the surrounding soil at planting time and again over the newly formed, well-grown tubers on 13 Aug. The pots were kept outside until 5 Oct. when they were brought indoors to dry out. The tubers were lifted very late in the season (10 Nov.) and stored till February; all (143) remained sound.

Field trials. Tubers (2–5 in. long) were uncovered and treated, while attached to the parent plants in the field, as follows: (*a*) inoculated internally with pure culture; (*b*) external inoculation of unbruised tubers with a suspension of spores and hyphae; (*c*) inoculated as in (*b*) immediately after the thin skin had been removed in several places. After inoculation each tuber (50 per series) was marked, re-covered with soil, lifted several weeks later and stored in new boxes. The dates of inoculation and lifting are given in Table 3. The haulms were green at inoculation time except where otherwise stated, and the *p*H of the soil was 5·7 in 1941, 5·2 in 1942, and 5·1 in 1943.

Controls, treated similarly but not inoculated, remained sound. In addition detached mature tubers were treated and inoculated as in series (*a*) and (*c*) except that in the latter the tough skin was removed with a file. These tubers were buried in the trial plot soil, and almost all of them developed dry rot thus showing that the fungus was viable and the soil conditions favourable for infection.

TABLE 3. *Dates of inoculation and lifting*

	Ninetyfold		Majestic		Doon Star	
Trial no.	Inoculated	Lifted	Inoculated	Lifted	Inoculated	Lifted
1	2. vii. 41	7. viii. 41	31. vii. 41	20. x. 41	—	—
2	9. vii. 42	13. viii. 42	23. vii. 42	29. ix. 42	—	—
3	5. vii. 43	12. viii. 43	18. viii. 43*	5. x. 43	18. viii. 43*	5. x. 43
4	19. viii. 43†	28. ix. 43	—	—	—	—

* Haulms partly yellow. † Haulms dead.

The results showed that no dry rot developed before or after lifting except in internally inoculated tubers in 1943, when eight tubers of Ninetyfold (Trial 4), three of Majestic and twenty-five of Doon Star were diseased when lifted and a few more of each variety developed dry rot in storage. The results of the pot and field trials indicate that little, if any, infection occurs in ordinary field crops before lifting. This conclusion was supported by other field trials in which the soil was contaminated at planting time by placing a diseased tuber in contact with each seed potato; the crop was healthy when lifted in August and remained so in storage. Crops grown in contaminated soil on Cheshire farms were healthy at digging time. Pethybridge & Lafferty (1917) reported that dry rot had not been observed in tubers still attached to the plant nor at digging time.

In trials 3 and 4 in Table 3, failure to establish infection when the skin was removed was not due to the tubers being non-susceptible since internal inoculation caused dry rot. The failure may be partly due to rapid healing of superficial wounds. The occurrence of dry rot in tubers internally

inoculated in August 1943 is interesting because of the failure of similar inoculations in July 1941–3.

Inoculation of newly dug tubers. Since little or no infection occurs before lifting, attempts (extending over three seasons) were made to infect freshly dug tubers in July and August. All the details cannot be published, but the treatment and results of one experiment are given in Table 4.

Discussion

Sources of infection. The fungus causing dry rot is frequently present in field soils in Cheshire and in soil on seed tubers imported from Scotland and Northern Ireland or stored in boxes. Other sources of infection include seed boxes, used sacks, loft sweepings, and diseased tubers contained in boxes,

Table 4. *Development of dry rot in freshly dug tubers, var. Ninetyfold*

Treatment of tubers	Date examined	No. tubers developing dry rot when stored in*		
		Moist chamber	Paper bags	Field soil outdoors
Dug and internal inoculation: 5. vii. 43	6. ix. 43	0	24	0
3. viii. 43	27. viii. 43	15	22	24
Dug, skin removed in several places, and inoculated with spore suspension: 5. vii. 43	6. ix. 43	0	4	0
3. viii. 43	27. viii. 43	21	24	14

* Twenty-eight tubers per series.

In each season infection was established and developed rapidly in July and August, but the amount varied considerably under different storage conditions (July test, Table 4); in most tests more infection occurred under dry conditions but occasionally the reverse happened.

In 1943, var. Majestic, King Edward, Redskin, Doon Star and Dunbar Standard developed dry rot when newly dug tubers (lifted at the usual times for the area) were internally inoculated and stored in paper bags.

A brief account of certain field trials (which will be published in full later) is given here because the results provide evidence on the time of infection. At Warburton Experimental Station, Cheshire, tubers of Ninetyfold were dug, inoculated with a spore suspension, bruised and clamped (959 tubers) or stored in boxes (268 tubers) on 19 July. On 1 Sept. the loss in the clamp was 37 % and in the boxes 38 %. In another test approximately 300 tubers of each of the varieties Arran Pilot (dug 15 July), Ulster Monarch and Catriona (dug 17 Aug.) and Doon Star (dug 1 Oct.) were inoculated with a spore suspension at digging time, then bruised and stored in boxes; all were severely attacked, the loss ranging from 18 to 31 % 8 weeks after digging. On nine farms in Cheshire tubers of Ninetyfold were 'standard' bruised at digging time (July 1943) and stored in boxes; dry rot appeared in September and was severe on seven farms (17–50 % loss) in October. These results are not exceptional nor are they dependent on seasonal conditions because similar results were obtained on six farms in 1942.

The results of the laboratory and field tests show that many varieties are susceptible at digging time and that serious infection may occur under farm conditions soon after lifting.

clamps or sacks. The effect of using contaminated knives, and the contamination of seed after treatment with fungicides, are not included in this discussion.

Where the field soil is contaminated, this, in the writer's opinion, is the main source of infection. Little, if any, relief may be expected by cleaning hampers, boxes, lofts, or sacks since these will soon be recontaminated.

Where the field soil is not contaminated it is difficult to assess the relative importance of the other sources of infection because this depends upon the number of tubers likely to be contaminated, the degree of contamination, and especially upon the treatment of the tubers at the time of, or subsequent to, contamination (varietal susceptibility is omitted here; the discussion deals with susceptible varieties). For instance, contamination will be widespread when (*a*) a contaminated loft containing seed is swept, (*b*) many diseased tubers occur in the boxes, (*c*) tubers are placed in a contaminated sack, (*d*) grading and packing produce from a clamp containing much dry rot, but almost all the sound tubers in (*a*) and (*b*) are likely to remain healthy so long as they are undisturbed, whereas those of (*c*) and (*d*) may develop dry rot because the contamination is often accompanied by, or is followed by, much bruising.

Time of infection. It has been shown that little, if any, infection occurs before lifting, and that serious loss may occur soon after lifting. Nevertheless, it has been observed repeatedly that externally inoculated, or naturally contaminated, tubers remain practically sound in boxes and in clamps when handled carefully; this lack of infection is not due to immaturity nor to conditions being unfavourable for infection, since bruised tubers under similar

conditions developed severe dry rot. Infection of unbruised tubers occurs, as shown by Pethybridge & Lafferty (1917) and in this paper, but this is unlikely to cause extensive loss; no information is available on the time of infection in these cases. The conclusion drawn from trials and observations is that, for serious outbreaks at least, the time and amount of infection of susceptible contaminated tubers after lifting are determined largely by the time and degree of bruising. Further evidence to support this generalization (to which exceptions possibly occur) will be given in a later paper.

Seed treatment. Two results obtained in this work (*a*) that the soil on healthy seed potatoes is often contaminated, (*b*) that little or no infection occurs before lifting, suggest that the disease may be controlled by suitable seed treatment (Foister, 1940; Foister & Wilson, 1943). If the above conclusion concerning the time of infection is valid, seed treatment need not always be carried out at digging time (a busy time for the farmer) as is often advised, and is probably unnecessary, where bruising is slight or absent. These practical points are being investigated.

I am indebted to Dr T. W. Walker, Agricultural Advisory Department, Manchester University, for the soil moisture and *p*H determinations.

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The prevention of seed-borne diseases of flax by seed disinfection

II. Comparison of the dusting, short wet and fixation methods of treatment

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Suitable organo-mercury preparations applied by the short wet method of seed treatment and organo-mercury powders, of the type used for treating cereal seeds, fixed to the seed with water by the fixation method of treatment, gave as satisfactory control of seedling blight and better control of stem break and browning than a proprietary dry fungicide containing tetra-methyl thiuram disulphide (Nomersan) applied at the rate of 12 oz./cwt. of seed.

Treatment of seed heavily infected with *Polyspora Lini* using an efficient fungicide resulted in highly significant increases in crop and fibre yields, while in the case of seed heavily infected with *Colletotrichum Lini* similar treatments significantly increased the yield of fibre but not the crop yield. Some treatments significantly increased the crop and fibre yields even when the seed used was only slightly contaminated with *P. Lini* and *C. Lini*. The results presented support the suggestion that effective treatment of the seed may produce an increase of fibre not directly attributable to the prevention of seedling blight or stem break and browning but which may be explained by a general beneficial effect due to seed disinfection.

The results obtained suggest that no treatment consistently affected crop establishment in the field.

In studying the prevention of seed-borne diseases of flax by seed disinfection it has been shown by the writers (Muskett & Colhoun, 1943) that a proprietary dust (Nomersan) containing tetra-methyl thiuram disulphide as its active constituent provides almost complete control of seedling blight caused by *Colletotrichum Lini* (Westerd.) Toch., and some control of stem break and browning caused by *Polyspora Lini* Laff. In addition, evidence was produced to show that a suitable soluble organo-mercurial (Ceresan U.564) applied by the short wet method provides very satisfactory control of both diseases. It was also indicated that organo-mercury dusts of the type used with cereal seeds gave satis-

factory results when adfixed to the seed with water or separated milk. Further, the results suggested, in at least one case, that the effective treatment of flax seed produces an increase of fibre not directly attributable to the prevention of seedling blight or stem break and browning. During 1942 a study of these problems was made with the object of comparing the different methods of treatment and determining whether a general beneficial effect can be ascribed to seed disinfection apart from the prevention of the diseases in question.

Choice of seed samples

Over 1000 samples were examined for the presence of seed-borne parasites by the Ulster Method (Muskett & Malone, 1941), and from among these two were selected, one being mainly contaminated with *P. Lini* and the other with *C. Lini*, both being almost free from any other known parasite. A third sample believed to be free from contamination with any parasitic organism was chosen, but when the bulk was examined it was found to be very slightly infected with *P. Lini* and *C. Lini*. The contamination was not considered to be sufficient to interfere with the suitability of the sample for the work.

Disinfectants used

The proprietary compounds used together with available details regarding their composition are as follows:

Material	Active ingredient	Metallic mercury (%)
Nomersan	Tetra-methyl thiuram disulphide	—
Ceresan 1875A	Organically combined mercury	1·5
Ceresan experimental	,, ,,	3·0
Ceresan U.564 (German manufacture)	,, ,,	3·5
Ceresan U.564/2/B1 (British manufacture)	,, ,,	3·5

Technique employed for evaluation

The technique for the treatment of flax seed by the three methods under consideration and for the evaluation of seed disinfectants for preventing attack by *P. Lini* or *C. Lini*, both in the laboratory and in the field, together with the procedure for the determination of crop and fibre yields, have already been described (Muskett & Colhoun, 1942, 1943). In this investigation field trial plots measuring 4×6 yd. ($3{\cdot}66 \times 5{\cdot}5$ m.) were used, each experiment being laid down in quadruplicate.

In order to study the effects of seed treatment on crop establishment, seed was sown by hand at a rate equivalent to 80 lb./acre in drills measuring 12 ft. (3·66 m.) long and spaced 6 in. (15 cm.) apart. About 4 weeks after sowing counts were made of the healthy and diseased seedlings in two drills sown with non-disinfected or disinfected seed from each sample. In the case of seed heavily contaminated with *P. Lini* further counts were made from two drills per treatment after the stem break phase of the disease had developed.

Effects of disinfection upon seed contaminated with *Polyspora Lini*

The seed used was of the variety Liral Crown; 18·8 % was contaminated with *P. Lini* and 6·4 % with *C. Lini*. Sowing was carried out on 12–13 May and the crop was pulled on 21 Aug.

The treatments employed, the results of the laboratory examination of treated seed, the mean values obtained for the incidence of the seedling, stem break and browning phases of attack by *P. Lini* in the field, together with the mean crop and fibre yields, are given in Table 1. In laboratory tests all treatments showed substantial control of *P. Lini*, Nomersan being the least effective. This result is in agreement with the field observations which show that Nomersan is inferior to any of the other treatments in preventing the occurrence of each of the three phases of the disease. All treatments significantly increased the crop and fibre yields above that of the untreated plots. Eight of the treatments significantly increased the crop yield over that given by the Nomersan treated seed, but in the case of only two treatments, Ceresan U.564, 8 % at 40 ml.* and Ceresan U.564/2/B1, 8 % at 30 ml./500 g. of seed was this increase in crop yield reflected to a significant amount in the yield of fibre. Only one significant difference in the crop yields exists between the plots sown with seed treated with different organo-mercurials although in a few cases such differences are apparent in the fibre yield.

Owing to considerable variations in the numbers of seedlings developing in drills sown with equal weights of seed receiving the same treatment, and to the fact that the counts were only made for two drills per treatment on each occasion, it is impossible to reach a definite conclusion as to whether any treatment produced a real effect upon crop establishment. There appeared, however, to be a tendency for treatments by either the short wet or fixation methods employing 40 ml. of liquid per 500 g. of seed to reduce the numbers of seedlings and older plants. This is not supported by results obtained and statistically examined in 1941 when a similar experiment was made employing drills 10 yd. (9·17 m.)

* 40 ml./500 g. = 0·9 gal./cwt.

TABLE 1. *The prevention of stem break and browning* (P. Lini) *by seed disinfection*, 1942

Seed sample used—Liral Crown

Treatment	Rate of application per 500 g.	Laboratory test (500 seeds examined) % infected seeds	Field test Maximum attack = 10 Seedling phase	Stem-break	Browning	Crop yield Per plot (lb.)	Crop yield Per acre (cwt.) (calculated)	Fibre yield Per plot (lb.)	Fibre yield Per acre (cwt.) (calculated)
Untreated	—	18·8	10	10	10·0	17·69	31·8	0·90	1·6
Nomersan	3·35 g.	9·8	6	8	10·0	**22·41**	40·3	**1·61**	2·9
Ceresan (1875 A) Fixed with water	3·35 g. 30 ml.	8·1	3	3	1·7	**26·13**	47·0	**1·71**	3·1
Ceresan (1875 A) Fixed with water	3·35 g. 40 ml.	8·4	1·5	3	1·6	**25·72**	46·3	**1·76**	3·2
Ceresan experimental Fixed with water	3·35 g. 30 ml.	3·8	0·2	2	1·1	**26·63**	47·9	**1·73**	3·1
Ceresan experimental Fixed with water	3·35 g. 40 ml.	3·2	0	2	1·4	**27·78**	50·0	**1·92**	3·5
Ceresan (U. 564) 6 %	40 ml.	8·4	2	4	0·9	**26·31**	47·4	**2·10**	3·8
Ceresan (U. 564) 8 %	30 ml.	8·4	1	3	1·2	**27·88**	50·2	**2·03**	3·7
Ceresan (U. 564) 8 %	40 ml.	6·4	1	2	1·1	**29·41**	52·9	**2·34**	4·2
Ceresan (U. 564/2/B1) 6 %	40 ml.	1·8	0·2	2·5	0·6	**25·25**	45·5	**1·99**	3·6
Ceresan (U. 564/2/B1) 8 %	30 ml.	2·8	1	2	0·9	**27·03**	48·7	**2·22**	4·0
Ceresan (U. 564/2/B1) 8 %	40 ml.	1·8	0	2	0·5	**26·07**	46·9	**1·87**	3·4

Statistical analysis

Yield of straw:

Analysis of variance Z (calculated for treatment) + 0·85557, 0·1 % point 0·6966 ($n_1 = 11$, $n_2 = 33$).
S.E. for difference between pairs of mean values ± 1·82.

Yield of fibre:

Analysis of variance Z (calculated for treatment) + 0·66648, 1 % point 0·5298 ($n_1 = 11$, $n_2 = 33$).
S.E. for difference between pairs of mean values ± 0·27.

Mean values for treatments showing significantly greater yields than that for the control are given in heavy type.

long in quintuplicate. In this case none of the following treatments (per 500 g. of seed) affected the number of seedlings to a significant amount when counts were made 5 weeks after sowing: Ceresan U. 564, 4 % at 40 ml.; Ceresan U. 564, 8 % at 30 ml. and at 40 ml.; and Nomersan 3·35 g.*

As far as the counts of plants in drills exhibiting the seedling or stem break phases of the attack by *P. Lini* are concerned the results obtained show that the percentage number of infected plants for each treatment agrees well with the observations made in the corresponding plots.

EFFECTS OF DISINFECTION UPON SEED CONTAMINATED WITH *COLLETOTRICHUM LINI*

One seed sample of the variety Liral Dominion was used containing 31·2 % of the seeds contaminated with *C. Lini* and 1·6 % with *P. Lini*. The seed was sown on 8–12 May and the crop was pulled on 21 Aug.

The treatments employed in this experiment were the same as in that dealing with seed contaminated with *P. Lini*. In Table 2 are given the results obtained from the laboratory examination of seed, the mean values obtained for the incidence of the seedling phase of attack by *C. Lini* in the field and the mean values for yields of crop and fibre.

It will be seen from Table 2 that in the laboratory experiments Ceresan U. 564/2/B1 at all the rates used gave as good control of *C. Lini* as Nomersan, whereas all the other treatments were inferior to Nomersan. In the field trials all treatments gave very good control of the disease and were equally effective as Nomersan in reducing seedling infection. The crop yield was not influenced by treatment but all treatments with one exception, when compared with the control, significantly increased the yield of fibre. Treatment with Ceresan 1875 A (3·35 g. fixed with 30 ml. of water) or Ceresan experimental (3·35 g. fixed with 40 ml. of water) produced crops which yielded significantly less fibre than those treated with Ceresan U. 564 (6 % or 8 % at 40 ml.). Treatment with Ceresan 1875 A (3·35 g. fixed with 30 ml. of water) also produced crops yielding significantly less fibre than those from seed treated with Nomersan at the 3·35 g. rate.

* 3·35 g./500 g. = 12 oz./cwt.

TABLE 2. *The prevention of seedling blight* (C. Lini) *by seed disinfection*—1942

Seed sample used—Liral Dominion

Treatment	Rate of application per 500 g.	Laboratory test (500 seeds examined) % infected seeds	Field test Maximum attack = 10 seedling phase	Crop yield: Per plot (lb.)	Crop yield: Per acre (cwt.) (calculated)	Fibre yield: Per plot (lb.)	Fibre yield: Per acre (cwt.) (calculated)
Untreated	—	31·2	10·0	24·31	43·8	1·70	3·1
Nomersan	3·35 g.	1·4	1·25	28·50	51·3	**3·27**	5·9
Ceresan (1875 A) Fixed with water	3·35 g. 30 ml.	6·8	0	26·85	48·3	2·39	4·3
Ceresan (1875 A) Fixed with water	3·35 g. 40 ml.	12·2	0·25	29·19	52·5	**3·16**	5·7
Ceresan experimental Fixed with water	3·35 g. 30 ml.	2·2	0	26·63	47·9	**2·74**	4·9
Ceresan experimental Fixed with water	3·35 g. 40 ml.	3·0	0	27·38	49·3	**2·65**	4·8
Ceresan (U. 564) 6 %	40 ml.	7·8	0	30·19	54·3	**3·43**	6·2
Ceresan (U. 564) 8 %	30 ml.	10·6	0·5	28·19	50·7	**3·06**	5·6
Ceresan (U. 564) 8 %	40 ml.	3·6	0	30·78	55·4	**3·45**	6·2
Ceresan (U. 564/2/B1) 6 %	40 ml.	0·6	0	28·53	51·4	**3·12**	5·6
Ceresan (U. 564/2/B1) 8 %	30 ml.	1·0	0	26·44	47·6	**2·77**	4·9
Ceresan (U. 564/2/B1) 8 %	40 ml.	0·4	0	27·13	48·8	**2·96**	5·3

Statistical analysis

Yield of straw:

Analysis of variance Z (calculated for treatment) −0·05747, 5 % point 0·3749 ($n_1 = 11$, $n_2 = 33$).

Yield of fibre:

Analysis of variance Z (calculated for treatment) +0·58778, 1 % point 0·5298 ($n_1 = 11$, $n_2 = 33$).
S.E. for difference between pairs of mean values ±0·389.

Mean values for treatments showing significantly greater yields than that for the control are given in heavy type.

TABLE 3. *Disinfection of seed not heavily infected with any known parasite*—1942

Seed sample used—Liral Prince

Treatment	Rate of application per 500 g.	Laboratory test (500 seeds examined) % seeds infected with: *P. Lini*	Laboratory test (500 seeds examined) % seeds infected with: *C. Lini*	Field test Maximum attack = 10: Seedling blight (*C. Lini*) Seedling phase	Field test Maximum attack = 10: Browning (*P. Lini*) Browning phase	Crop yield: Per plot (lb.)	Crop yield: Per acre (cwt.) (calculated)	Fibre yield: Per plot (lb.)	Fibre yield: Per acre (cwt.) (calculated)
Untreated	—	0·2	1·5	10	10	25·38	45·7	1·99	3·6
Nomersan	1·68 g.	0·2	1·0	0	7	26·63	47·9	2·31	4·2
Nomersan	3·35 g.	0	0	0	8·5	**30·89**	55·6	**2·98**	5·4
Ceresan (1875 A) Fixed with water	3·35 g. 40 ml.	0	0	0	3·5	27·69	49·8	2·35	4·2
Ceresan experimental Fixed with water	3·35 g. 40 ml.	0	0	0	2·5	23·19	41·7	1·50	2·7
Ceresan (U. 564) 8 %	30 ml.	0	0·2	0	7·0	26·69	53·4	**2·62**	4·7
Ceresan (U. 564) 8 %	40 ml.	0	0·4	0	5·0	**30·66**	55·2	**2·80**	5·0

Statistical analysis

Yield of straw:

Analysis of variance Z (calculated for treatment) +0·66238, 5 % point 0·4894 ($n_1 = 6$, $n_2 = 18$).
S.E. for difference between pairs of mean values ±2·10.

Yield of fibre:

Analysis of variance Z (calculated for treatment) +0·85391, 1 % point 0·6950 ($n_1 = 6$, $n_2 = 18$).
S.E. for difference between pairs of mean values ±0·30.

Mean values for treatments showing significantly greater yields than that for the control are given in heavy type.

The results obtained by counting the numbers of diseased and healthy seedlings in drills sown at the standard rate showed that all treatments gave good control of *C. Lini*, the average number of infected seedlings for any treatment not exceeding 0·8 % as compared with a corresponding figure of 32·6 % for the untreated seed. Crop establishment was not influenced by seed treatment.

Effects of treatment upon seed almost free from contamination with any parasite

One seed sample of the variety Liral Prince was employed having 0·2 % of the seeds contaminated with *P. Lini* and 1·5 % with *C. Lini*. Sowing was carried out on 4–5 May and the crop was pulled on 20 Aug.

The results obtained from the laboratory examination of the seed together with the mean values of the incidence of the seedling phase of attack by *C. Lini* and of the browning phase of attack by *P. Lini* are given in Table 3. Since the seedling and stem break phases of the attack by *P. Lini* were almost absent no observations were recorded. In Table 3 are also shown the mean crop and fibre yields.

It is seen from Table 3 that seedling blight was absent from all plots sown with treated seed although it was present to a very small extent in the control plots. Browning developed to varying degrees in all plots but was not prevalent in the controls.

The crop and fibre yields were significantly increased by two treatments, Nomersan (3·35 g.) and Ceresan U.564 (8 % at 40 ml.) while treatment with Ceresan U.564 (8 % at 30 ml.) significantly increased the fibre but not the crop yield.

Little infection with *P. Lini* occurred in the seedlings in drills sown at the standard rate but about 6 % of those developing from untreated seed were affected with *C. Lini*. All treatments completely eliminated infection with *C. Lini*. None of the treatments employed produced a real effect upon crop establishment.

Discussion

The results presented in this paper confirm the previous findings that the disinfection of flax seed materially assists towards cleaning it from contamination with *P. Lini* and *C. Lini*. In the case of *P. Lini*, as pointed out in an earlier paper (Muskett & Colhoun, 1943), Nomersan is only partially effective in securing control. This has again been demonstrated and it is shown that treatment by the short wet or fixation methods using organo-mercurial preparations gives much better results although they do not entirely eliminate the parasite. All the treatments employed gave substantial control of *C. Lini* and Ceresan U.564/2/B1 proved as efficient in reducing infection as Nomersan which has given highly satisfactory results.

In the case of stem break and browning (*P. Lini*) all the treatments employed significantly increased the yields of crop and fibre. The greater efficiency of organo-mercurial preparations applied by the short wet or fixation methods in reducing contamination of the seed with *P. Lini* is reflected in the yields. While a considerable number of treatments gave significantly better results for crop yield than did Nomersan, only two treatments, viz. 8 % solutions of Ceresan U.564 and Ceresan U.564/2/B1, using 0·9 and 0·67 gal./cwt. respectively, brought about a significantly higher yield of fibre than Nomersan applied at the rate of 12 oz./cwt. In spite of the fact that from an economic point of view the yield of fibre is the important consideration, it should be realized that as browning spreads so rapidly after its first appearance and may exercise a great effect in reducing the yield of fibre it is unwise to use the fibre yield as the sole means of evaluating the efficiency of a fungicide for the control of this disease. All the results available must be considered in conjunction with those for fibre yield if the value of the fungicide is to be truly assessed. There is a possibility that a fungicide which gives satisfactory results in experimental plots might be even more satisfactory when used on a commercial scale for crops not grown in close proximity to any which are badly infected.

As in the case of results obtained in 1940 and 1941 treatment of seed heavily contaminated with *C. Lini* did not significantly affect the crop yield although most treatments significantly increased the yield of fibre. The results for 1942 indicate that in controlling *C. Lini* most of the treatments employed do not influence the yield of fibre to a greater extent than dressing with Nomersan at the rate of 12 oz./cwt.

It has recently been shown (Colhoun, 1944) that Ceresan U.564 applied by the short wet method and Nomersan used by the dusting method are more effective than organo-mercurial powders adfixed to the seed in bringing about the control of *Botrytis cinerea*, the cause of grey mould of flax.

The results obtained from the experiment made with seed very slightly infected with *P. Lini* or *C. Lini* are of interest since it is shown that the yield of fibre in crops produced by such seed was significantly increased by using the following treatments (per cwt. of seed): Nomersan 12 oz.; Ceresan U.564 8 % at 0·9 or 0·67 gal. The last treatment did not significantly influence the crop yield. This indicates that beneficial results may be obtained from the disinfection of seed not heavily infected with either parasite and the results obtained from this experiment support the contention that disinfection may carry with it a benefit other than that which can be attributed to the control of specific seed-borne parasites.

The results obtained during this study suggest that no treatment consistently influences the crop establishment under field conditions.

When flax seed which has been treated with Nomersan by the dusting method is broadcast using a 'fiddle' sower, as is the practice in Northern Ireland, the powder may cause a certain amount of inconvenience. This is completely eliminated by the use of the short wet or fixation methods. As compared with the short wet or dusting method the fixation method involves two distinct processes—the dusting of the seed with the powder followed by its fixation to the seed and for this reason it may not be so popular in practice as the other two methods.

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The 'phloem necrosis' virus disease of tea in Ceylon

II. Field observations and effect on yield

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(With 4 Text-figures)

After a brief discussion of the methods and accuracy of diagnosis of the 'phloem necrosis' disease in the field, the results of plot records taken over a number of years are presented. These are compared with the figures supplied from estates for the removal of non-productive diseased bushes, which is undertaken as a routine measure of control and regeneration of affected fields. Taking all the evidence together a frequency of at least 25 % diseased bushes is suggested for the aggregate acreage of tea above 6000 ft. At lower elevations no reliable estimate is possible.

Despite the pronounced effect on individual bushes there appears to be no general tendency for the yield of affected estates to decline. Two estates, believed to be representative, are dealt with in which a decline in yield occurred from 1929 to 1930, followed by a recovery some 4 or 5 years later. The fields judged to be most heavily affected in each of the two properties, respectively, show a decline in yield relative to the estate as a whole, not exceeding 19 lb. made tea per acre per annum, which is only 3·3 % of the mean yield during the last 10 years. The great variability in individual bush yields is stressed, and the conclusion is drawn that the elimination of unproductive bushes and their replacement by supplies of a type which, so far, has remained unaffected (although possibly 'carrying' the virus) will more than counteract the effect of the continued and gradual deterioration of the necrotic bushes remaining.

The distribution of diseased bushes in the field is irregular, and records from observation plots have mostly given positive results on examination by Cochran's geometric series 'aggregation' test and the χ^2 test for 'neighbour infection'. The rate of spread of the disease in the different areas is highly variable, and doubling of the incidence of infection may occur in periods ranging from $3\frac{1}{2}$ to less than 1 year.

Two 'rogueing' experiments are described, the results of which lend further emphasis to the importance of bush-to-bush spread. The course of the disease in these experiments has been expressed by means of the Verhulst-Pearl logistic curve, whence it appears that complete rogueing of all 'necrotic' and 'suspected' bushes at 6-monthly intervals has reduced the rate of spread to about one-third of the value for the unrogued plots.

'Phloem necrosis' of tea in Ceylon was fully described in the first paper of this series (Bond, 1944*a*). In the present paper an account is given of observations and experiments in the field whereby the infectious nature of the disease is further substantiated and its probable effect on yield may be estimated. The question of control in the field is also considered.

Diagnosis in the field

The problem in the field is to combine rapidity with accuracy of diagnosis. In experiments involving several hundred bushes which have to be recorded during a single day an average of half a minute or less is all that can be allowed for the individual bush, and diagnosis is practically confined to the detection of necrosis in the petiole of a few leaves plucked from different regions of the bush. Advanced cases are recognizable at a glance, but some others may easily be overlooked. On the other hand, 'false necrosis' (Bond, 1944*a*) may be confusing and in some instances tends to increase the apparent incidence of the disease.

In practice, three categories, 'healthy' (H), 'suspected' (S), and 'necrotic' (N), are recorded. 'Suspected' are either those bushes which are diseased in appearance but in which no necrosis can be detected, or those in which necrosis is found in one or two leaves only, without confirmation in other leaves, and in the absence of external symptoms sufficient to warrant a 'necrotic' record. The use of this category as, in fact, an early stage of the disease is justified by the high percentage, up to 95 % in the experiments hitherto recorded, of 'suspected' bushes subsequently marked 'necrotic'. The proportion of 'suspected' bushes is not usually more than about 20 % of the 'suspected' and 'necrotic' bushes together, and may be less than 10 % in severely affected areas.

The accuracy of the recording as a whole may best be judged by the proportion of 'suspected' and 'necrotic' bushes which are subsequently marked 'healthy'. Over a period of 2–3 years, this proportion has varied between the limits of 5 and 30 %. The higher values, representing an accuracy of 70–80 % only, have been obtained on St Coombs estate (elevation 4600 ft.), where certain fields are notoriously difficult to record and the incidence of the disease is in any case low, with a high proportion of 'suspected' records. At higher altitudes (6000 ft. and over), where most of the field work has been carried out, the accuracy of recording has usually exceeded 90 %. Since 1940, all field records have been taken by the writer personally, to reduce subjective error as far as possible. Where a comparison with earlier records has been necessary, substantial agreement in the standard of recording has been effected by suitable checking.

In view of the difficulties of diagnosis outlined above and dealt with at length in the previous paper, it has to be admitted that any record of the incidence of the disease is inevitably an estimate only of the actual state of affairs when the record was taken. Consequently, in following the progress of the disease through successive intervals of time, there are two methods of dealing with the results obtained. One is to take the records as they stand, the other is to use the *cumulative* total of 'necrotic' and 'suspected' bushes, i.e. to treat all N and S records as of equal value irrespective of whether they are confirmed subsequently or not. Without undertaking a full analysis of the available records for a total of more than 4000 bushes kept under observation for periods ranging from $2\frac{1}{2}$ to 6 years, it is not possible to assess fully the error introduced by the cumulative method. For instance, the interval of recording, sometimes 6 months, sometimes a year, sometimes 3 years, will appreciably affect the estimation of this error. The *cumulative* incidence of disease can alone be used, e.g. in 'rogueing' experiments, as described below. For other purposes, and except where noted to the contrary, the *actual* percentage of 'necrotic' and 'suspected' bushes, as given by the last available recording, is used.

Frequency in affected areas

It was previously stated that the total acreage affected by 'phloem necrosis' might reach 150,000 acres, the disease being of almost universal occurrence at elevations above 4000 ft. The severity of the disease increases with increasing altitude and appears to reach its maximum in the relatively few estates at 6000 ft. and over, representing some 8500 acres. Even here it is by no means every bush that is affected; in a given area the proportion of such bushes will vary according to the length of time that the disease has been present and the proportion of susceptible types, although, if allowance be made for the occurrence of symptomless 'carriers' (Bond, 1944*a*), the actual incidence of the disease may be considerably higher than appearances suggest. With this proviso, some idea of the frequency of the disease at different elevations may be obtained from two sources, namely, observation plots in the field, and the figures supplied by estates for removal of necrotic bushes.

Field plots. Observation plots have been maintained on St Coombs and other estates since the beginning of 1938. Usually, an average size of about 200 bushes has been aimed at, although for some purposes much larger areas have been recorded. The field plots as a whole fall into two groups, those from 4000 to 4600 ft. and those above 6000 ft. elevation, each group consisting of approximately 2000 bushes in all. The aggregate percentage of 'necrotic' and 'suspected' bushes in the two groups

(from records between 1939 and 1943) stands at 18·6 and 55·5 % respectively. The difference between these two figures gives a fair estimate of the general difference in the incidence of the disease at low and high elevations. The field plots have usually been put down in the most severely affected areas of the estates in question. Usually, in the early days of the investigation, an estate reported a necrotic locality or sent in a severely necrotic specimen, and a plot was established, it being realized only later that the disease was present elsewhere in a less severe form. Thus, the figures given are not fully representative. An experiment on St Coombs illustrates this point where, in a 42-acre field, the incidence of 'phloem necrosis' was estimated from rows selected more or less at random and from three plots put down in known necrotic parts of the field. The rows, six in all, varying from 72 to 364 bushes in length (according to the shape of the field), gave 14·0 % diseased bushes, whereas the percentage from the plots was 26·5.

Estate removal figures. The only recommendation at present made to estates affected by 'phloem necrosis' is that non-productive bushes should be removed (Gadd, 1939). Actually, the elimination of severely necrotic bushes is undertaken on a considerable scale only above 6000 ft., and here it is practised to different degrees of thoroughness by different estates. In any case, it is a considerable drain on the available labour supply. A few examples only can be given to illustrate the magnitude of the problem.

Estate 'A'. This estate of 398 acres has systematically removed non-productive bushes since 1933. At that time the condition of the bushes was not recognized as 'phloem necrosis', but there is every reason to believe that it was so, and that the great majority of the bushes removed have, in fact, been necrotic. In the 10 years 1933–42, removals have totalled 184,518 bushes, or the equivalent, at 3000 bushes/acre, of some 15 % of the entire acreage of the estate. The fields are 'rogued' chiefly at pruning time, so that the removals may be expressed as a percentage for the acreage pruned each year, namely, 110 acres. On this basis, the *yearly* removals average 5·6 % of the acreage pruned.

Estate 'B'. Removals for the 6 years 1937–42 have totalled 117,151 bushes from 268 acres—15 % of the estate as a whole, or about 8 % yearly of the acreage pruned.

Estate 'C'. Removals since 1938 have totalled 65,564 bushes, from 536 acres—4 % of the estate, or 4·2 % yearly of the acreage pruned.

Bushes judged to be non-productive by estates are, with few exceptions (at least, at the higher altitudes which are alone under consideration here), severely necrotic. Consequently, the estate removal percentages given above must be multiplied several times before they can be compared with the figures for *total* 'necrotic' and 'suspected' records obtained from the observation plots. Two 'rogueing' experiments, which are discussed later, may be used as an illustration of this. Here, in equal areas of some 600 bushes, removals according to rather strict estate standards during the last 3 years have accounted for 127 bushes, whereas the writer's tally of 'necrotic' and 'suspected' bushes during the same period has amounted to 419 bushes, i.e. 3·3 times as many as the estate figure. On this basis, the yearly removals from the three estates quoted above, considered in relation to the pruning acreage, may be regarded as at least the equivalent of a total incidence of some 20 % diseased bushes, a figure which, as will be shown subsequently, is subject to a fairly rapid increase as the disease spreads.

Taking both sets of figures together a conservative estimate of 20–25 % incidence of diseased bushes may be allowed for estates as a whole above 6000 ft. At the lower altitudes, no reliable estimate can be given owing to the high proportion of recent 'high *jât*' fields which are practically free from the disease, and to the irregular distribution of necrotic bushes in the affected fields, to which reference has already been made (Bond, 1944*a*, and see below).

Effect on yield

That severely necrotic bushes are rendered almost entirely unproductive can scarcely be called into question. Such bushes are usually delayed in recovery from pruning, they produce only dwarfed, curled leaves with but little flush suitable for plucking and appear to be progressively weakened until, frequently, they succumb to drought, root diseases, or other adverse influences. No information is available as to the length of time taken to reach this condition: the indications are that it may be many years. In addition, as already noted, the average necrotic field will include far more bushes which are infected but are scarcely or not recognizable as such externally. In fact, there are likely to be present bushes in all stages of the disease and of all grades of susceptibility from symptomless 'carrying' onwards. Normal, healthy tea bushes are themselves so variable in performance that no reliable estimate of the effect of 'phloem necrosis' in varying degrees of severity can be made, and the opportunity for detailed studies using clonal material is as yet lacking. The appearance of affected fields, and the fact that many estates consider it worth while to replace severely necrotic bushes, suggests that the disease, in the aggregate, must have a very appreciable effect on yield. Nevertheless, as is shown in the following paragraphs, this is by no means easy to demonstrate.

Data have been examined from various estates and, of these, only two will be discussed in detail. These are estates 'A' and 'B' of the previous section,

neighbouring properties which are among the most severely necrotic in Ceylon. Both estates were asked to furnish average yields/acre over a number of years for the estate as a whole and for the fields judged to be 'most' and 'least' necrotic respectively. Figures from an 'average' field are also available from estate 'B'. The crop from individual fields fluctuates according to the stage in the pruning cycle (see Fig. 1); consequently, all the figures as averages of both properties exhibit a similar fluctuation from a minimum in about 1921, rising to a maximum 8–9 years later and again falling to a minimum in 1934. Since that year, they have both increased up to the last year (1941) for which the running averages can be calculated. Thus, there is no evidence of any *progressive* decline in recent years, resulting from the progressive increase in the disease. The fluctuation generally may be due in part to

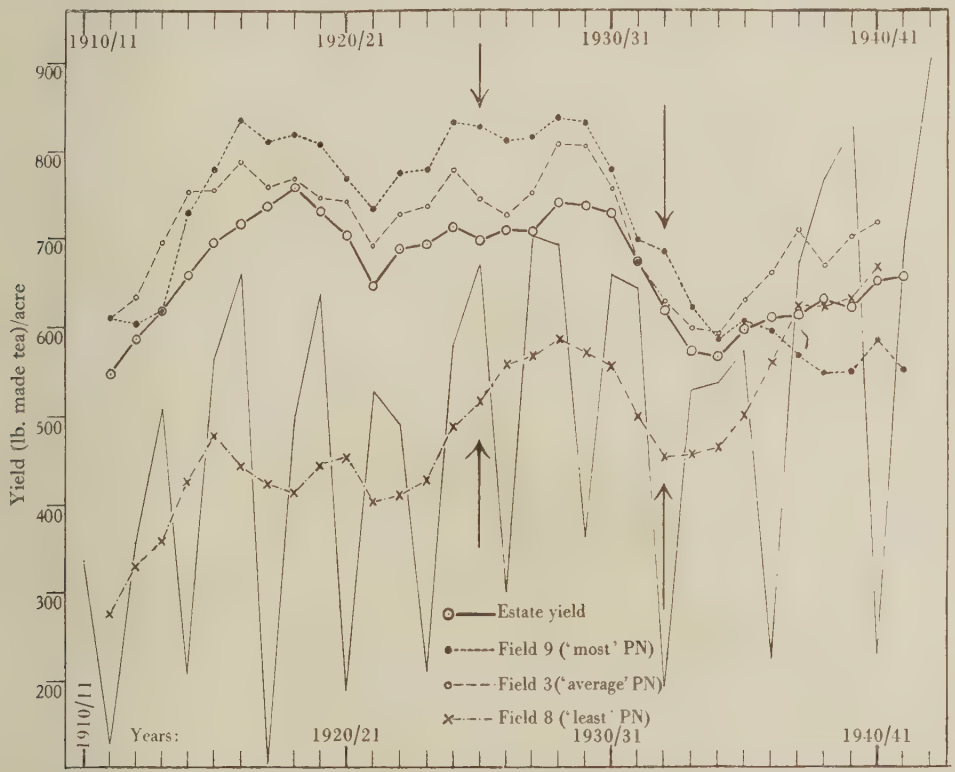


Fig. 1. Estate 'B'. Annual yields of estate as a whole and of individual fields, as running averages for the pruning cycle of 3 or 4 years. For field 8, the *individual* yields are also included, to show the extent of fluctuation within the cycle, the minima corresponding to the years of pruning. Arrows at 1925–6 and at 1932–3.

received have been converted into running averages for cycles of 3 or 4 years as the case may be. The method of investigation (see Figs. 1–3) has been to follow the trend of the (converted) field and estate yields as such and to examine the field yields considered as deviations from the yield of the estate as a whole, in each case. All yields and deviations, in the graphs, are in terms of lb. made tea/acre.

Data from estate 'B' were available from 1910 to 1911, those from estate 'A' from 1919. Considering the trends in yield of the two estates as a whole, it is apparent from Figs. 1 and 3*a* that the 3-year running climatic or other complex factors. In addition, there is reason to believe that the decline from 1918, for instance, was due to the shortage of fertilizers at the end of the last war. If the second decline, from 1929 to 1930, be attributed to the onset of 'phloem necrosis', then the subsequent increase can be viewed as evidence of the successful reversal of the decline through the policy of removal of necrotic bushes (see above) and their replacement by 'high *jât*' supplies which, so far, are showing no signs of becoming diseased.

Considering the fields individually, the situation

in estate 'B' (Fig. 1) is complicated by the fact that field 8 ('least' necrotic) has been until recently considerably below the estate average in yield. However, while showing much the same general fluctuation, it has fairly consistently *increased* relative to the estate and is at present more or less level with it at an annual yield of some 650 lb./acre. Field 9 ('most' necrotic), on the other hand, was consistently above the estate average until, after the 1934 minimum, it failed to recover, falling below the estate to a more or less constant yield of about 550 lb. Field 3 ('average' necrotic) is consistently and slightly above the estate average in yield.

On estate 'A' (Fig. 3*a*) the 'least' necrotic field, 2, has been consistently above the estate average, the 'most' necrotic field, 8, was originally slightly above the estate average, but fell below in 1927 and, as in the case of field 9 on estate 'B', failed to recover from the 1934 minimum.

The deviations of these fields from their estate averages, respectively, are shown in Figs. 2 and 3*b*. Study of the data as a whole suggests two periods as of special significance, viz. between 1925 and 1927, and 1932–3. The field deviations expressed as linear regressions (with their probability level by *t*-test) are given above in Table 1, as from each of these two dates.

Relative to the estate, the 'most' necrotic fields are declining in yield in both cases. However, the magnitude of this decline is not great, as may be judged from the fact that the highest value recorded in Table 1, namely, −19·0 lb./annum for field 9 on

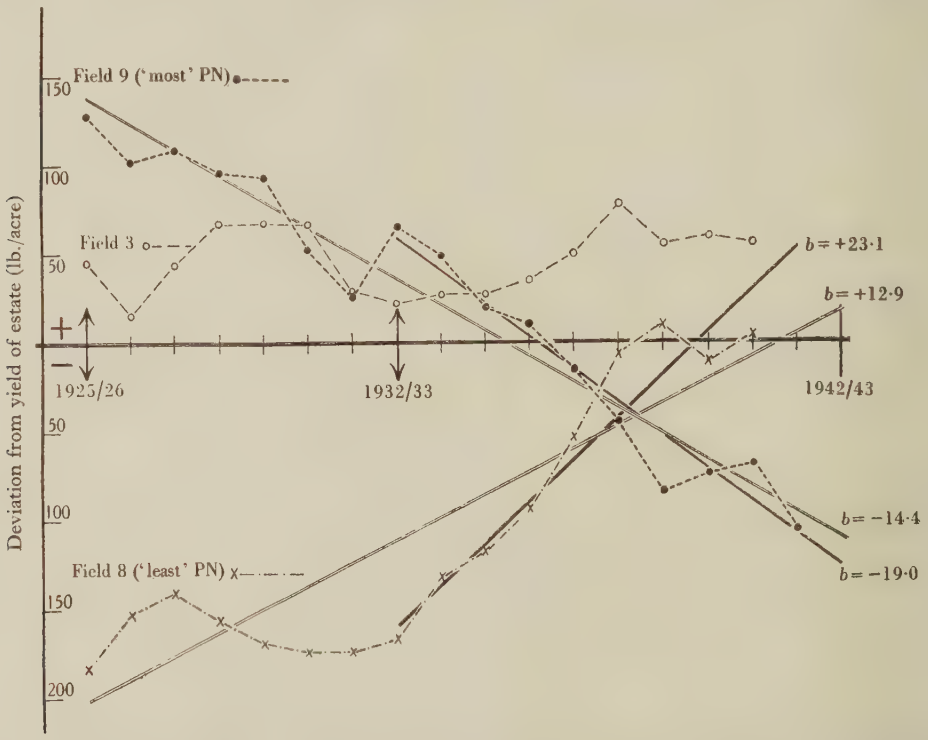
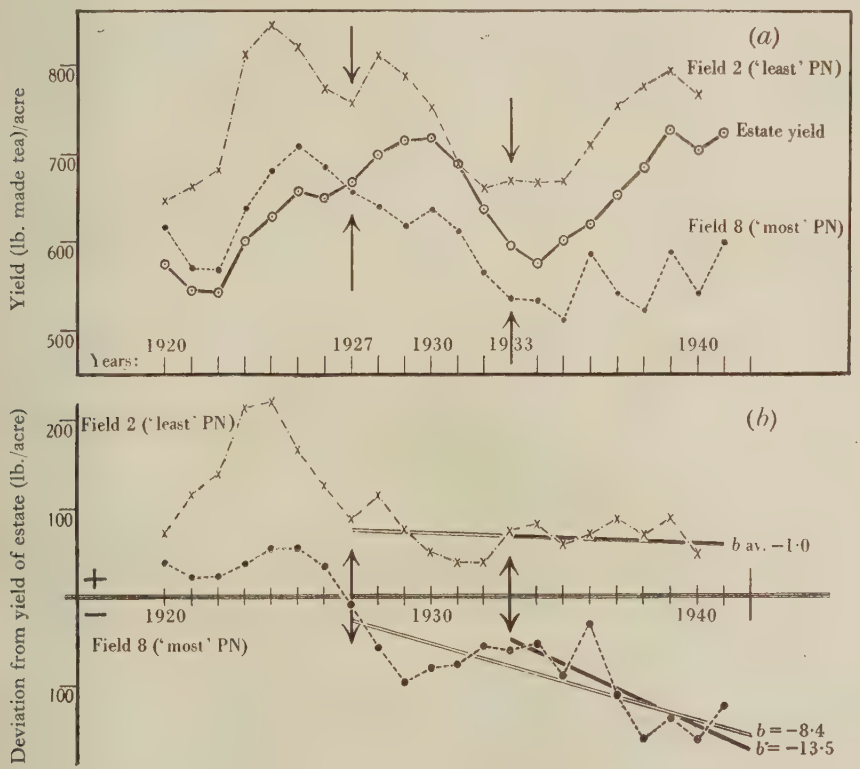


Fig. 2. Estate 'B'. Deviations of field yields from yield of the estate as a whole, with the calculated regressions from 1925 to 1926 and from 1932 to 1933. Values of *b* as in Table 1.

TABLE 1. *Regressions of field yields as deviations from estate (lb./acre/annum)*

	Estate 'B'			Estate 'A'		
Field	No.	Since 1925–6	Since 1932–3	No.	Since 1927	Since 1933
'Least' PN	(8)	+12·9 ($P<0·01$)	+23·1 ($P<0·01$)	(2)	−0·9 ($P>0·5$)	− 1·1 ($P>0·8$)
'Most' PN	(9)	−14·4 ($P<0·01$)	−19·0 ($P<0·01$)	(8)	−8·4 ($P<0·02$)	−13·5 ($P<0·1$)

estate 'B', is only 3·3 % of the mean annual yield for the period in question (i.e. since 1932–3). The decline, in this field, appears to be maintained: in field 8 on estate 'A', however, there is evidence (see Fig. 3*b*) that it has been arrested and may shortly be reversed, a fact which may also account for the low significance ($P<0{\cdot}1$ only) of the regression of −13·5 lb./annum since 1933. In *actual* yields, the decline of these necrotic fields appears to have been more or less reversed in estate 'A' since 1935, and single large area within which the majority of bushes may be diseased but in which numerous discontinuities, representing groups of healthy bushes, still persist. On the other hand, isolated diseased bushes occur from which the subsequent development of a patch may frequently be traced. Such a distribution in the field, which is of common occurrence among virus diseases, and is particularly noticeable in the case of perennial crops (e.g. Posnette, 1943), suggests a dual means of spread, viz. a discontinuous,



Figs. 3*a*,*b*. Estate 'A'. Annual yields (3*a*) and deviations from the estate yield (3*b*), as in Figs. 1 and 2.

at least arrested in estate 'B', since 1938–9. The comparison of the 'most' and 'least' necrotic fields therefore confirms the general impression derived from the study of the estate yields as a whole.

Manner and rate of spread

One of the most noticeable features of 'phloem necrosis' in the field is the tendency for the disease to occur in localized areas or patches. These may vary from a few feet to several yards in diameter and are of varying degrees of distinctness. Thus, several small patches may be loosely associated to form a more or less random dispersal affecting bushes relatively remote from the nearest centre of infection and, in addition, a continuous or bush-to-bush spread.

In order to obtain more precise information on this point, the field plot data were examined by the methods of Cochran (1936), principally the geometric series 'linear aggregation' test and the χ^2 'neighbour infection' test. The results are shown in Table 2. Both tests are significant in the majority of cases. The exceptions, to the aggregation test, include the two smallest plots, '4' and '6', and plot '10', which has the highest percentage incidence of

disease. The St Coombs plots, totalling 677 bushes, which show no significance for the 1943 aggregation test or for neighbour infection, 1939–43, actually comprise five small plots varying in size from 60 to 236 bushes, so that here too the failure to attain significance may be due to the unsuitability of the data. In most fields, the planting is too irregular for the tests to be applied across the rows as well as along them. This, however, was tried out in plot '8' and resulted in significant evidence for aggregation in 1940 and for neighbour infection, 1938–40. The same plot also gave a significant result from the binomial series test (Bond, 1944*a*) for aggregation within equal areas of nine bushes. The data as a whole amply confirm the visual impression of the localized distribution and bush-to-bush spread of 'phloem necrosis' in the field.

Table 2. *'Phloem necrosis' field records. 'Aggregation' and 'neighbour infection' tests*

Source of data	Total bushes	Year	Diseased (%)	*P* value for linear aggregation test	Neighbour infection test
Plot '1'	221	1939	5·1	$<0{\cdot}01$	—
St Coombs, rows	1134	1940	5·6	$<0{\cdot}01$	Significant ($P<0{\cdot}01$)
		1943	9·2	$<0{\cdot}01$	
Plot '3'	211	1940	6·2	$<0{\cdot}02$	—
Plot '4'	161	1940	8·7	$>0{\cdot}05$	—
Plot '5'	277	1940	11·2	$<0{\cdot}01$	Significant ($P<0{\cdot}01$)
		1943	18·8	$<0{\cdot}01$	
Plot '6'	164	1940	16·5	*	—
St Coombs, plots	677	1939	18·6	$<0{\cdot}01$	Not significant
		1943	30·3	$>0{\cdot}05$	
Plot '8'	985	1938	26·2	$<0{\cdot}01$	Significant ($P<0{\cdot}01$)
			53·2	$<0{\cdot}01$	
Plot '9'	292	1939	47·3	$<0{\cdot}01$	—
Plot '10'	209	1939	75·6	*	—

From the foregoing it will be apparent that the rate of spread of 'phloem necrosis' as determined from field-plot records will vary greatly according to the size and position of the plot and the age of the disease within the area chosen. For example, a small plot on the edge of an advancing 'patch' of the disease will give a high rate of spread; a plot with a single old patch, a low rate, which will increase as the plot is enlarged to include other, younger patches. Some idea of the rate of spread in different localities is given by the four sets of data in Table 1, to which the 'neighbour infection' tests were applied, viz. the St Coombs rows and plots and plot '5' at less than 5000 ft. elevation, plot '8' above 6000 ft. The first three of these conform closely to an annual geometric increase of 22 %, or to doubling of the incidence of disease every $3\frac{1}{2}$ years. The rate of increase as calculated from all available data has varied between this value and a maximum corresponding to more than 100 %, or to doubling in less than a year.

Rogueing experiments

In order to test the hypothesis of bush-to-bush spread more fully and at the same time to examine the effectiveness of the removal of non-productive bushes in checking the spread of the disease, a rogueing experiment was set up on estate 'B' (see above) in August 1940. The experiment consists of three plots of rather more than a hundred bushes, in each of three fields known to be more or less severely necrotic. The fields, all above 6000 ft. elevation, are about a quarter of a mile apart, but the plots in each field are as nearly as possible adjacent. The plots are approximately square, ten rows in width, and are not isolated in any way from the adjacent tea except where bounded by drains or paths.

Records have been taken at 6-monthly intervals beginning in September 1940. The three plots in each field received the following treatments, respectively, viz.:

A. Control, no bushes removed.

B. Removal of bushes judged to be 'non-productive' by estate standards.

C. Removal of all bushes marked 'suspected' or 'necrotic', by the writer.

The 'B' or estate rogueing is carried out by the Superintendent of the estate: in effect, it represents a more thorough treatment than the normal estate practice owing to the more frequent inspection, namely, once every 6 months instead of the usual interval of the 3-year pruning cycle. Bushes removed during the 3 years of the experiment have totalled 38, as compared with 181 from the 'C' plots.

Since the 'C' plots, in which every necrotic or suspected bush is removed immediately on marking as such, form the basis of comparison, all the results are expressed cumulatively, i.e. no allowance is made,

in the 'A' and 'B' plots, for bushes subsequently becoming 'healthy' after an initial 'diseased' record. In other words, in all plots, only the bushes which have remained consecutively 'healthy' from the first record onwards are regarded as such. The number of consecutively healthy bushes, subtracted from the plot totals successively, forms the simplest expression of the cumulative progress of the disease.

The results of the experiment up to the 7th record in September 1943, are shown graphically in Fig. 4.

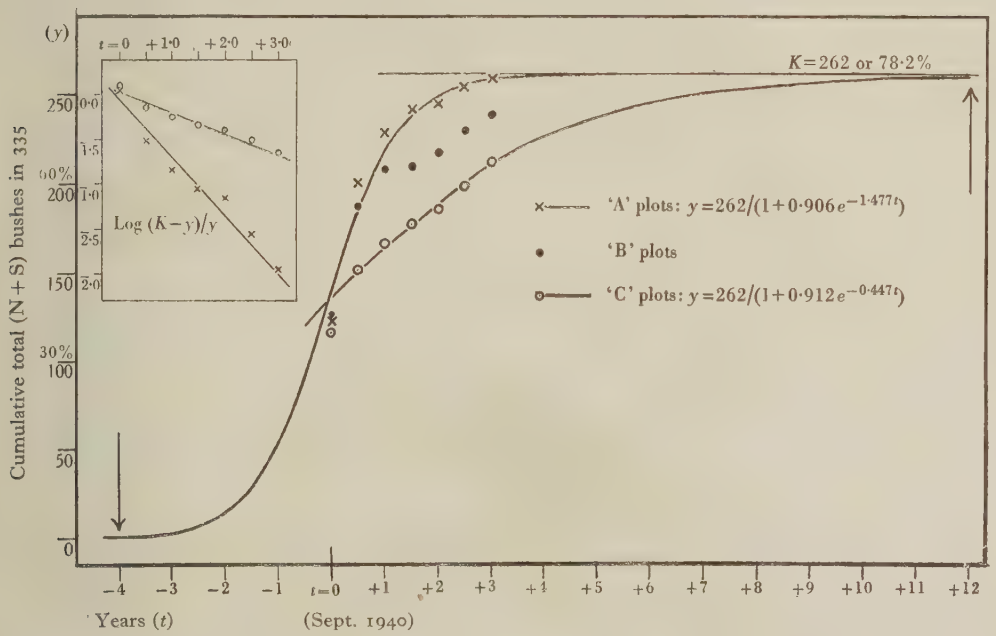


Fig. 4. The 'rogueing' experiment on Estate 'B'. The points show the cumulative incidence of 'necrotic' and 'suspected' bushes at 6-monthly intervals of time from $t=0$ in September 1940, for the three treatments, each being an average from three plots. The two curves correspond to the logistic expressions as computed with the aid of the log $(K-y)/y$ plotting, *inset*. The arrows mark the hypothetical origin of the disease in September 1936, and 'saturation' (C plots) in September 1952. No logistic curve has been constructed for the B plot data.

The data from the three fields have been combined. It will be seen that the 'A', 'B' and 'C' plots started approximately level with an average of 36 % 'necrotic' and 'suspected' bushes. Thereafter, the incidence of disease has increased, in the 'A' plots to the equivalent of 77 %, in the 'B' plots to 71 %, and in the 'C' plots to 64 %. Both methods of rogueing appear to have been effective in checking the rate of spread of the disease during the 3 years of the experiment.

Further, the data show that, at present, the disease is actually spreading *faster* in the rogued plots than in the unrogued. Thus, the new disease records during the past year have been as follows: 'A', 14; 'B', 20; and 'C', 26 bushes. In effect, the 'A' plots appear to be becoming saturated with the disease and the rogueing of the 'B' and 'C' plots has merely delayed the process so that these plots, being farther from their saturation level, are now showing a higher rate of increase in the incidence of disease.

A mathematical analysis has been attempted by means of the Verhulst-Pearl logistic formula $y=K/(1+Ce^{-rt})$, viewing the increase of the disease as an example of the growth of a population of diseased bushes up to the maximum, or upper asymptote (K), representing the total proportion of susceptible units in the area under observation. Recently, the use and properties of the logistic curve have been discussed by the writer in relation to the growth of the tea plant (see Bond; 1944*b*), so that no further details need be given here (see Pearl, 1940, where the method of computation is fully set out). For the purpose of fitting the curves, the 'A' plots can be considered to be approaching saturation at 262 bushes out of the total of 335 (or 78 %), and the same value can reasonably be assumed for the 'B' and 'C' plots, which are adjacent, comparable areas. On this assumption, the 'A' data can be graduated to the logistic expression $y=262/(1+0{\cdot}906e^{-1{\cdot}477t})$. The 'growth rate' 1·477 corresponds to an annual

geometric increase of 338 %, or to doubling of the incidence of infection every $5\frac{1}{2}$ months. That this high rate of increase is not, in fact, adhered to, follows from the 'damping' effect of the factor $K-y$, used for the logarithmic determination of r, as in the inset to Fig. 4 (Pearl, 1940). Similarly, the 'C' data (after correction to the same total of 335 bushes) agree with the expression $y=262/(1+0{\cdot}912e^{-0{\cdot}447t})$, whence it appears that the rate of increase, 0·4467, is now only about one-third of the value determined for the 'A' plots. In this case it represents a geometric increase of 53 % annually, or doubling of the incidence of infection in about $18\frac{1}{2}$ months. The 'B' data in turn, correspond to a logistic growth rate of 0·645, a geometric increase of 90 % annually, or doubling in 13 months.

The logistic representation of the data also enables a prediction to be made of the time of the ultimate 'saturation' of the plots and of the probable age of the disease within the area as a whole. Taking saturation, for practical purposes, to be the equivalent of 261 diseased bushes out of the asymptotic value of 262, this value will be reached, by the 'A' plots in March 1944 ($t=3{\cdot}5$) and by the 'C' plots in September 1952 ($t=12{\cdot}0$). Similarly, the theoretical origin of the disease at the first diseased bush is found to be in September 1936 ($y=0{\cdot}8$, $t=-4{\cdot}0$). In all probability, as has already been stated, the disease has been known in this district for many more years than this, which throws some doubt on the validity of the logistic expression for predictions based on the early part of the curve. On the other hand, it should be remembered that the calculations are based on the *cumulative* incidence of the disease, whereas the *actual* figures would be lower at each record after the first and would give a flatter curve with a correspondingly earlier estimate of the probable origin of the disease.

The experiment was later repeated by subdividing the large plot '8' of Table 2, which was on a neighbouring estate. Here again a total of more than 900 bushes was available and the same arrangement of nine plots with triple replication of the 'A', 'B', and 'C' treatments was adopted. The initial percentage incidence of the disease was considerably higher than in the original experiment, averaging 66 %. However, the probable saturation level is also somewhat higher and a value of 84 % has been postulated. Owing to the relatively narrow range over which the increase in the incidence of disease has been followed, the results are of less value than those of the original experiment and the fitting of the logistic curves is necessarily more liable to error. However, here again the potential rate of increase in disease in the 'C' plots has been less than one-third of the value computed for the 'A' plots. The 'B' treatment, in this experiment, has had scarcely any effect.

Taken together, the two experiments provide ample evidence of the effectiveness of *complete* rogueing in reducing the spread of the disease and suggest that *partial* rogueing may also be effective to a certain extent. The fact that rogueing, within these small and completely unprotected areas of tea, should have any effect at all, is considered to confirm the existence and relative importance of bush-to-bush spread as compared with random dispersal. From this point of view, the continued and gradual increase in the 'C' plots is the result of infection from outside, i.e. by random dispersal, whereas the more rapid increase and consequent earlier saturation of the unrogued 'A' plots is due to the additional, unimpeded bush-to-bush spread within the plots themselves.

Discussion

The main features of 'phloem necrosis' in the field, as now established, may be summarized in the following paragraphs. The position starts from the recognition of the disease as a serious one, potentially capable of causing a progressive deterioration of affected bushes until they virtually cease to be productive.

The disease is evidently spreading within the known affected areas although, so far, there is no satisfactory evidence of any tendency to increase the total range by extending to areas below about 4000 ft. elevation. From this level, however, the incidence of the disease, its average intensity and, as far as can be determined, its rate of spread, all tend to increase up to the highest estates in Ceylon, above 6000 ft., where the *total* incidence of the disease in all recognizable forms is at least 20–25 % and may be much higher. At this high elevation, the rogueing experiments, both in severely necrotic areas, point to a probable 'saturation' level of about 80 % diseased bushes. Among the existing, old tea, the 'low *jât*' types have principally become affected, but there is evidence also of a tendency towards 'deterioration of *jât*' as a result of infection. The most encouraging feature in the field, so far, has been the virtually complete absence of the disease among the younger, 'high *jât*' supplies, although the experiments reported previously (Bond, 1944*a*) indicate that some, at least, of these may actually be symptomless 'carriers' of the virus.

The only control measure so far feasible on estates (except for complete replanting, which has been tried on a small scale only) is the removal of bushes judged to be so severely diseased as to be non-productive, and their replacement by supplies. In practice, this has proved to be a considerable expense and, naturally, absorbs a good deal of available labour. It has been carried out, on most of the estates principally affected, for a number of years, and appears to have produced satisfactory results as evidenced by the trend of estate yields. In fact, the *absence* of any marked decline in yield resulting from 'phloem necrosis' appears paradoxical and has been the

subject of comment in planting circles, particularly in the case of certain fields known to be heavily necrotic which continue to give high yields of upwards of 1000 lb./acre. From the figures quoted for estates 'A' and 'B' above (which are believed to be representative), it might be argued, for example, that the decline in yield of the 'most' necrotic fields relative to their estates respectively is the result of the heavy removals from those fields rather than of any direct effect of the disease itself. Actually, from the fields in question, removals (total) during the last 7–10 years have averaged 30 % as compared with 15 % from the estates as a whole, so that there might be considerable justification for this view.

At least two lines of evidence are relevant here, as to the effect of such removals on yield. *First*, 'thinning' by itself is known to result in an increase in size of the bushes remaining which will give, therefore, a proportionately greater yield. Pfältzer (1940), for instance, showed that, in Java, the yield *per bush* increased with increasing planting distance, the increase being almost proportional to the spacing between the limits of about 6700 to 1800 bushes/acre. Garretsen (1929) previously gave examples of the effect of unselective thinning in which the removal of 50 % of the bushes reduced the yield by some 12 % only on the average. *Secondly*, this effect will be all the more pronounced where, as in the present case, the bushes 'thinned' out are known, or can be assumed, to be below average productivity. This has been stressed recently, in Java by Hoedt & Schoorel (1938), and in Ceylon by Tubbs (1939), who states that the 50 % best bushes may account for from 60 to 80 % of the yield in an average field. Detailed examination of Tubbs's data showed that, in one such area of 1513 bushes, the top 10 and 25 % of the bushes in order of individual merit gave 23 and 48 % respectively of the total yield, the bottom 10 and 25 % gave only 3 and 10 % respectively, whilst the bottom 50 % accounted for only 30 % of the yield.

The two estates 'A' and 'B' for which figures are given, despite the high incidence of necrosis and heavy removals during recent years, have both been increasing in yield since about 1934: the relative decline in yield of their 'most' necrotic fields has averaged barely 2 % per annum, or 6 % for the 3-year pruning cycle, as compared with an average removal each cycle of 13 % of the total bushes. Furthermore, while the incidence of the disease is still increasing, and the proportion of 'non-productive' bushes awaiting removal is, apparently, as high as ever, there is distinct evidence from the yield figures that in these 'necrotic' fields, as in the estates generally, the decline in yield that might be attributed to 'phloem necrosis' has, in fact, been arrested. On the whole, the rather conflicting evidence may justifiably lead to two conclusions, viz.: (1) The bushes removed have at least been well below average productivity if not entirely unproductive and, in all probability, this condition has been of long standing, i.e. the effects of the disease, on the individual bush, have been gradual and progressive. (2) The supplies put in to replace these bushes are gradually coming into bearing and, while they continue to remain free from the disease, may be expected to contribute an increasing proportion of the total yield. In all probability they will more than counteract the effect of the continued deterioration of the remaining affected bushes.

A further direction in which the 'rogueing' of non-productive bushes may prove advantageous is in the reduction of the subsequent rate of spread of the disease through the elimination of sources of infection. In ordinary estate practice, however, this is highly problematical. Complete rogueing, as in the two experiments described above, is obviously impracticable on a field scale and, as already noted, the 'estate' rogueing in these experiments was more thorough than that usually adopted, if only because of more frequent inspection. The importance of the rogueing experiments lies not so much in the practical result achieved as in the evidence which they afford of the occurrence and relative preponderance of bush-to-bush spread. The 'neighbour infection' tests by themselves are not conclusive for, as Cochran (1936, p. 66) points out, a positive result may only mean that certain small areas which were previously attractive to, for example, the infective insect vectors, were remaining so. On the other hand, a positive result from the rogueing experiments at least emphasizes the importance of the necrotic bush as such, rather than of the locality in which it is situated, and the most reasonable conclusion from this is to regard it as a source of infection to the bushes in its immediate vicinity. That the beneficial effect of rogueing has been demonstrated at all, in these small and completely unisolated plots, is in itself evidence of the relative importance of bush-to-bush spread as compared with 'random' dispersal, since, by the latter method, all the plots would be equally liable to infection from outside.

The mechanism of spread remains unknown. Bush-to-bush spread could conveniently be accounted for by mechanical transmission during the operations of pruning and plucking, but this, as noted by Bond (1944*a*), seems unlikely on *a priori* grounds, nor has it received any support so far from further experiments as suggested on that occasion. The possibilities of spread under ground, or through the agency of insects of relatively limited powers of flight, remain for further investigation together with the problem of random dispersal over greater distances.

As on the previous occasion, I am indebted to Dr C. H. Gadd for placing at my disposal the data obtained prior to my appointment in February 1940,

and for his subsequent advice and helpful criticism. It is apparent that this field side of the investigation could not have been carried out without the willing and active co-operation of Estate Superintendents. To those who have assisted in this way, and particularly to those whose hospitality I have enjoyed on numerous occasions, my grateful thanks are due.

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The transmission of the virus of the 'woodiness' disease of passion fruit (*Passiflora edulis*) by single leaf grafts

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(With Plate 11)

A method is described of transmitting the virus of the 'woodiness' disease of passion fruit by means of single leaf grafts. The grafts can be made from one species to another in the genera *Passiflora* and *Tacsonia* and from these genera to species of *Adenia* and vice versa.

The 'woodiness' disease of passion fruit has proved to be a limiting factor in the successful cultivation of the crop in Kenya. The crop is grown commercially for the most part in two widely separated districts, the Trans Nzoia in the north-west of the colony and in the Sotik district, some 150 miles to the south. Each of these areas has its own characteristic form of the disease, and each form has been shown to be caused by a distinct virus (Nattrass, 1939, 1940). There are, in addition, at least three other distinct types or strains which have since been demonstrated from other parts of the colony.

The disease as it occurs in the Trans Nzoia closely resembles, and may be identical with, that first described by Noble (1928) in Australia. The fact, however, that the Trans Nzoia form occurs only in one district and that it has been freely transmitted to indigenous species of *Adenia*, suggests that the disease may have spread to the cultivated passion fruit from some wild host plant. There are believed to be no indigenous species of *Passiflora* in Kenya. The virus is not carried in the seed and, as far as is known, there have been no importations of living plants in recent years.

As all attempts at direct control proved to be ineffective, it seemed that the only hope of saving the industry lay in the finding or breeding of an immune or highly resistant variety. The work, started in 1938 but discontinued after 1941 owing to war conditions, consisted of testing a number of species and varieties of *Passiflora*, *Tacsonia*, and certain indigenous species of *Adenia* with the virulent and highly infective Trans Nzoia virus. At the outset a collection was made of as many of these species as could be found in East Africa. In addition, collections were obtained largely through the East African Agricultural Research Institute, Amani, of *Passiflora* and *Tacsonia* species from most of the tropical and sub-

Fig. 1

Fig. 2

Fig. 3

Fig. 5

Fig. 4

tropical botanic gardens of the world. It is believed that most of the known species were eventually received. Except for a study of the different types of virus encountered, all the routine testing was done with the Trans Nzoia type. It may be noted, however, that infection with one of the milder but highly invasive types of virus gives no immunity from, or resistance to, the more virulent type.

Of the number of species which have been tested, only two or three appear to be highly resistant or immune, but further confirmation is needed. There are, however, varying degrees of tolerance and recovery, and some species are apparently symptomless carriers.

Although the Trans Nzoia virus can be transmitted by the insertion of juice or tissue, as was first demonstrated by Storey (*in litt.*), the method is not entirely satisfactory in that absence of symptoms is not necessarily an indication that the plant is immune, unless a considerable number of inoculations is made. Moreover, other types have not been so transmitted.

At first, tests were carried out by means of stem grafts. Either a young infected plant was inarched to the plant to be tested, the stem of the plant being severed when union has occurred, or the scion, an apical or lateral shoot 4–6 in. long, was approach-grafted at about the third internode at any convenient point on the stock. The basal part of the scion was immersed in 1–2 in. of water in a short test-tube suitably supported either with the graft binding on the stock itself, or its support.

This method of grafting is generally successful, but is cumbersome and wasteful of material. It is, moreover, not possible to tell with certainty if union has taken place without either undoing the binding or removing the tube of water.

To obviate this time lag and to conserve material, a trial was made of single leaves as the scion. It was found that not only did union readily occur but also that transmission of the virus in all forms of the disease invariably followed union. The leaves remain in an assimilating condition for 2–3 months after being grafted.

The method is simple, a leaf of medium size is removed with at least 1 in. of petiole. This is cut or scraped to an elongated wedge. On the stock a downward cut is made, almost parallel to the stem, opening up a thin strip of cortex. The petiole is then inserted as far as it will go and the whole bound up with a thin strip of 'Slipknot' pure rubber tape. This is of the semi-tacky variety which is sufficiently elastic to exert slight pressure and effectively closes the wound, making an airtight cover. With large leaves it is advisable to cut down the area of the leaf surface to reduce any tendency to wilt unless the plants are kept in an atmosphere of high humidity. It makes no difference whether the dorsal or the ventral surface of the leaf is uppermost (see Plate 11).

The advantages of this method, where a large number of plants have to be tested or when a detailed study of the virus in a single plant has to be made, are obvious. One infected vine will supply material for a number of grafts over a long period. The graft can be made at any point on the stem of the stock. No supports are required and no special precautions are necessary to keep the graft moist. More than two hundred such grafts have been done in an ordinary greenhouse, and leaves have been grafted on to mature vines in the open during the rainy season (see Plate 11). It is possible to tell within a comparatively short time, usually 24 hr., whether or not the graft has taken: wilting of the leaf occurs if it has failed. By this method all the types of virus so far encountered have been freely transmitted. No difficulty has been experienced in making interspecific grafts in the genera *Passiflora* and *Tacsonia* and in grafting leaves of *Passiflora* spp. on to certain indigenous species of *Adenia* and in passing the virus to this genus from *Passiflora*.

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EXPLANATION OF PLATE 11

Fig. 1. Leaf of *Passiflora edulis* grafted on to *P. subpeltata*: 30 days after grafting.

Fig. 2. *P. ligularis* grafted 30 May 1940 with leaf of *P. edulis* infected with severe Trans-Nzoia strain. Photo 16 Aug. 1940. Leaf graft at *A*, severe symptoms above point *B*.

Fig. 3. Leaf of *P. edulis*, infected with mild but highly invasive strain of virus, grafted on to plant no. 143 on 11 July 1939. Photo 12 Aug. 1939.

Fig. 4. Terminal of plant no. 143. Photo 29 Aug. 1943, 50 days after grafting leaf shown in Fig. 3.

Fig. 5. Leaf of *P. caerulea* grafted on to mature vine of *P. subpeltata* in the open, after 14 days.

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A two years' survey of the potato aphides in the Northern Agricultural Advisory Province

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(With 1 Text-figure)

The role of aphis vectors in the deterioration of potato stocks is well known, and any effort to maintain healthy (virus-free) stocks of potatoes depends on the degree of their isolation from these vectors. In practice this isolation depends on geographical, climatic, and biological factors; of these the biological factors are fundamental, as it is only from their study that the significant geographical and climatic factors can be determined.

The present work consists of a survey made during 1942 and 1943, in the Northern Agricultural Advisory Province. Special attention is paid to overwintering, and the prevailing weather conditions at the time of the spring migration.

Introduction

In addition to taxonomic work, population studies, field experiments and laboratory experiments on the potato aphides have been undertaken. The experimental work has been mainly concerned with the ability of the various species to act as vectors of virus diseases, and the study of the conditions necessary for flight. The present paper is concerned only with the study of populations.

Population studies have been made in north Wales by Walton (1925), Davies (1932, 1934), Thomas & Vevai (1940), Jacob (1941), Thomas & Jacob (1943), and Whitehead (1943) quotes some of Davies's unpublished data. The detailed ecological work, started by Dr Maldwyn Davies and continued by his successors, has resulted in the accumulation of a great deal of important information regarding the year to year changes in the population of potato aphides in north Wales. In addition, Davies visited east and North-east Scotland from 25 July to 6 Aug. 1936, completing a considerable number of observations. One of the main results of this survey was to indicate the desirability of a more intensive work in these parts of Scotland. Staniland (1943) carried out a survey in Devon and Cornwall from 1937 to 1941, and compares the behaviour of the potato aphides in the warm moist climate of south-west England with their behaviour in the cool moist climate of North Wales. Detailed investigations on the biology of *Aphis rhamni* B.d.F., in the eastern counties, have been described by Doncaster (1943). Loughnane (1940) has made population studies in Eire, and on the continent, Heinze & Profft (1938), Heinze (1939), in Germany, and Ossianilsson (1943), in Sweden, have made similar studies. In addition, Ossianilsson refers to work in Denmark by Larsson (1942). In the Eastern Provinces of Canada, Gorham & Burnham (1935) and Gorham (1940, 1941, 1942*a*, 1942*b*, 1943) describe observations which have been continued since 1934.

Much of this work has been directed to the determination of the critical populations above which the maintenance of healthy stocks of seed potatoes is not a practicable proposition; and also, the factors which influence the establishment and development of such populations. Davies (1934) stipulated 20–100 *Myzus persicae* Sulzer per 100 leaves as 'the absolute maximum, which in an early season would be permissible'. In Germany, 15 *M. persicae* per 100 leaves is regarded as the critical level. In Canada, extensive use is made of the aphis survey for the purposes of seed-potato inspection.

The Northern Agricultural Advisory Province forms a particularly interesting area for the study of the potato aphides, in its contrast between the cool dry climate of the north-east coast and the cool moist climate of the north-western counties, in addition to having many districts which are specialized on account of mountainous or industrial conditions. Added interest is provided by the existence of areas which have proved to be suitable for the production of high-grade seed potatoes, without detailed knowledge of the potato aphides in the province.

Methods and scope of the survey

The aphides under consideration are *Myzus persicae* Sulzer, *Aphis* (*Doralis*) *rhamni* B.d.F., *Macrosiphum solanifolii* Ashm. and *Myzus pseudosolani* Theob. *Aphis* (*Doralis*) *frangulae* Kalt., which on the continent is known to migrate from *Rhamnus Frangula* L. to potatoes, was not found on either of these hosts.

In 1942 farms were visited in widely separated parts of the Province, in order to gain a general knowledge of the distribution of the aphides under consideration. During the winter 1942–3, winter hosts were located and examined. In the spring 1943, Dr F. T. Bennett kindly provided a list of farms in seed potato-growing areas, which would be

suitable for the purposes of the survey. Farms were chosen in each of the four northern counties, mainly for their relationship to established seed potato-growing areas, and the distribution of winter hosts, in particular savoy cabbage (*Brassica oleracea* var.), *Rhamnus catharticus* L. and *R. Frangula* L. The centres chosen are shown in Fig. 1. They may be roughly classified into the following main districts:

(1) Northumberland: I Tweedside (3 farms).
II Rothbury & Otterburn (3 farms).
III Hexhamshire (3 farms).
IV Lower Tyne area (2 farms).
V Cockle Park.

(2) Durham: I Lower Tees (1 farm).
II Midde Tees (2 farms).
III Weardale (2 farms).

(3) Cumberland: I Longtown (1 farm).
II Carlisle (2 farms).
III Penrith (5 farms).

(4) Westmorland: I Ambleside (3 farms).
II Grange (1 farm).

Seed potato-growing areas, the main districts where market-garden crops are grown on a farm scale, and the known distribution of *Rhamnus catharticus* and *R. Frangula* are shown in Fig. 1. In 1943 an effort was made to visit each centre three times in order to obtain data in mid-June, mid-July and mid-August.

Most of the counts were made on 100 leaves picked at random from the middle part of 100 plants (Davies, 1934). Occasionally three leaves, top, middle and lower, were taken from fifty plants at random (Thomas & Jacob, 1943). In 1943 several fields were sampled by each method on the same day; the traverse used for the first count being walked over in the reverse direction to make the second. Davies's method, which was quicker, was sufficient to obtain an estimate of the general trend of the population, but the second method is preferable if it is desired to obtain a detailed knowledge of the distribution on the plants, of the different species. In none of the counts made was any great difference found between the data derived from each method, when they were reduced to the common basis of the number of aphides/100 leaves. As a rule, only fifty leaves were counted when the population was high. On two occasions the number of aphides on flower heads was counted.

Details of the survey in 1942 and 1943

The 1942 and 1943 observations are summarized in Tables 1 and 2 respectively. In these tables the index figure for each species of aphis (i.e. the number of aphides/100 leaves), and the percentage of leaves infested by each species are shown. The latter figure gives an indication of the spread of the aphides through the crop, during the season. The figures for each species are given in the following order: *M. persicae*, *M. solanifolii*, *A. rhamni*, *M. pseudosolani*, the index figure being expressed as a fraction of the percentage figure. Farms visited in 1942 and 1943 are denoted by capital letters, and those visited for the first time in 1943 are shown by arabic numerals, as in Fig. 1.

The number of aphides on flower heads was counted on two occasions:

2 July. Farm 2. 228 *M. persicae*, and 1180 *M. solanifolii* on 100 heads.

24 July. Farm D. 36 *M. persicae*, and 1808 *M. solanifolii* on 100 heads.

It will be seen that at the time of flowering the greater part of the *Macrosiphum solanifolii* population, and a considerable part of the *Myzus persicae* population in these crops, occurred on the flower heads. This observation is in keeping with the fact that *M. solanifolii* generally shows a preference for the upper part of the plant.

For the interpretation of the 1942 and 1943 data several factors need to be taken into consideration. They may be summarized as follows:

(*a*) The history of the aphis population in the previous year, particularly with regard to the establishment of hibernating stages.

(*b*) Actual observations of overwintering.

(*c*) The distribution of winter hosts.

(*d*) The weather conditions at critical periods.

(*e*) The activities of predators and parasites.

Where (*a*) is concerned no information is available prior to 1942. Data are available for (*b*), (*c*) is indicated in Fig. 1, and (*d*) is considered in some detail in connexion with migration conditions.

Over-wintering

(I) *Myzus persicae* Sulz. On the Continent, peaches and nectarines are regarded as the most important winter hosts, although Heinze & Profft (1940) consider that hibernation may occur on Brassicae in the milder parts of western Germany. Ossianilsson (1943) comments on the difficulties of overwintering in Sweden, where winter hosts are not abundant. In Canada, eggs are laid on *Prunus nigra* Ait. (Gorham, 1942*b*), and Loughnane (1940) says that overwintering always occurs on Brassicae in Eire. Jacob (1941) confirmed Davies's (1934) opinion that savoy cabbage (*Brassica oleracea* var.) is the most important winter host in North Wales. As in North Wales, peaches and nectarines are not considered to be important winter host plants in the Northern Province. Weather, in relation to overwintering, is discussed below.

In the mild winter of 1942–3, hibernation as apterous and alate viviparous females on savoys, was

Fig. 1. Sketch-map showing the farms visited during the 1942 and 1943 potato aphis survey and the known distribution of the winter host plants of *Aphis rhamni* B.d.F.

TABLE 1. *Number of aphides/100 leaves, and percentage of leaves infested by* Myzus persicae, Macrosiphum solanifolii, Aphis rhamni, *and* Myzus pseudosolani, *in* 1942 (*see text*)

Farm and approx. altitude (ft.)	Date	No. of aphides/100 leaves and percentage of leaves infested			
		M. persicae	*M. solanifolii*	*A. rhamni*	*M. pseudosolani*
A, 40	2 June	0	0	0	0
	2 July	2/1	0	0	0
	24 ,,	9/4	9/2	3/2	1/1
B, 300–400	8 June	0	0	0	0
	9 July	0	0	190/12	0
	10 Sept.	Plants dead, but farmer stated that infestation was very heavy in Aug.			
C, 0	10 June	0	0	0	0
	15 July	1/1	2/1	14/3	0
	28 Aug.	187/41	104/32	115/13	4/4
D, 100	11 June	0	0	12/5	0
	9 July	0	0	125/20	0
	10 Sept.	2/2	1/1	10/8	0
E, 300–350	15 June	0	0	4/–	7/–
	30 July	11/5	0	18/5	2/2
F, 30	16 June	0	0	0	0
	30 July	172/31	60/17	16/3	10/2
G, 320	23 June	0	0	0	0
	15 July	0	0	0	1/1
	27 Aug.	345/48	7/3	190/18	8/6
H, 1100	14 July	Several *Aphis rhamni*			
I, 200	15 July	2/2	0	0	0
J, 100	24 July	12/6	2/2	0	0

TABLE 2. *Number of aphides/100 leaves, and percentage of leaves infected by* Myzus persicae, Macrosiphum solanifolii, Aphis rhamni, *and* Myzus pseudosolani, *in* 1943 (*see text*)

Farm and approx. altitude (ft.)	Date	No. of aphides/100 leaves and percentage of leaves infested			
		M. persicae	*M. solanifolii*	*A. rhamni*	*M. pseudosolani*
Tweedside	2 July	50/30	9/6	0	2/2
A, 40	3 Aug.	169/52	68/31	0	1/1
	3 Sept.	2/2	0	0	0
1, 50	3 Aug.	52/31	21/13	0	4/3
	3 Sept.	2/2	3/1	0	0
2, 200	2 July	29/15	2/2	0	0
	3 Aug.	37/21	17/15	0	0
Rothbury and Otterburn	30 June	16/6	3/1	0	0
3, 600	27 July	31/8	76/33	0	0
	27 Aug.	4/4	4/4	0	0
4, 340	30 June	5/4	0	0	0
	27 July	18/10	19/8	0	0
5, 550–600	30 June	21/4	11/3	0	1/1
	29 July	72/20	61/29	0	0
	27 Aug.	1/1	8/3	0	0
6, 600	30 June	3/2	2/2	0	0
	29 July	40/22	78/34	0	14/2
Hexhamshire	1 July	2/2	8/4	0	1/1
9, 600	28 ,,	30/21	32/21	0	0
	7 Sept.	10/5	5/4	0	0
10, 950–1000	1 July	5/3	0	0	0
	28 ,,	29/18	8/6	0	0
	7 Sept.	3/3	1/1	0	0
11, 750–800	1 July	24/10	4/4	0	0
	28 ,,	37/24	80/30	0	0
	7 Sept.	0	4/2	0	0

TABLE 2 (*cont.*)

Farm and approx. altitude (ft.)	Date	No. of aphides/100 leaves, and percentage of leaves infested			
		M. persicae	*M. solanifolii*	*A. rhamni*	*M. pseudosolani*
Lower Tyne	7 July	129/41	38/21	0	2/2
7, 150	9 Aug.	24/15	10/7	0	0
8, 340	5 July	326/62	356/74	0	3/3
	26 ,,	366/82	666/98	0	0
	2 Sept.	4/3	0	0	0
Cockle Park	5 July	22/11	4/6	0	2/1
G, 320	31 ,,	74/30	165/55	0	0
	14 Aug.	5/5	6/5	0	0
	30 ,,	5/4	0	0	0
Lower Tees	29 June	29/20	115/35	0	16/5
D, 100	24 July	196/68	144/60	56/2	2/2
	24 Aug.	17/15	28/19	0	1/1
Middle Tees	29 June (I)	10/8	42/32	0	0
B, 350–400	24 July (I)	118/98	810/98	8/4	4/4
	(II)	294/80	800/80	0	10/4
	24 Aug. (I)	8/5	6/5	0	0
14, 450	29 June	9/4	2/1	0	2/1
	24 July	120/50	77/37	3/1	2/2
	24 Aug.	16/16	19/13	8/4	1/1
Weardale	8 July	2/1	10/5	0	0
12, 950	6 Aug.	28/16	72/44	0	0
	6 Sept.	34/21	47/29	0	0
13, 6–700	8 July	38/14	5/5	0	0
	6 Aug.	40/28	51/33	0	0
	6 Sept.	5/4	14/10	0	0
Longtown	21 June	0	0	0	0
15, 300	21 July	27/7	1/1	0	0
Carlisle and Penrith	21 June	1/1	0	0	0
F, 30	21 July	78/8	58/28	0	3/2
	18 Aug.	27/16	9/4	0	0
16, 200	13 July	16/12	49/12	0	0
	18 Aug.	18/16	93/47	0	1/1
17, 600	13 July	0	0	0	0
18, 400	25 June (I)	2/0·5	3/0·25	0	1/0·5
	19 July (I)	23/4	17/2	0	1/1
	(II)	99/26	11/6	0	0
	18 Aug. (I)	47/25	30/15	0	0
	(II)	49/26	20/15	0	7/3
19, 550	13 July	9/3	4/2	0	0
20, 600	19 July	0	0	0	0
	21 Aug.	29/22	9/5	0	0
21, 500	26 June	2/2	0	0	0
	19 July	19/11	5/3	4/1	0
	21 Aug.	27/21	35/23	6/2	0
22, 500	13 July	7/6	0	0	0
Westmorland	22 June	11/9	1/1	2/1	2/2
23, 200	20 July	79/37	9/7	11/1	0
	19 Aug.	79/33	15/8	38/6	0
24, 350	22 June	16/10	2/2	0	0
	20 July	17/11	6/4	4/1	0
	19 Aug.	20/14	37/14	0	0
25, 600	22 June	4/3	0	0	0
	20 July	6/4	2/2	0	0
	19 Aug.	30/22	34/14	0	2/2
26, 15	23 June	12/7	0	1?/1	3/2
	20 July	63/26	35/21	42/4	9/1
	19 Aug.	36/12	15/11	5/3	0

probably widespread. Some details are shown in Table 3. In addition, *Myzus persicae* was found in small numbers on other brassicae.

TABLE 3. Myzus persicae *on savoys*, 1942–3. *No./100 leaves*

Date	Centres in south Northumberland			
	1	2	3	4
13 Oct.	—	—	48	—
23 ,,	692	—	—	—
27 ,,	—	122	—	—
13 Nov.	487	—	—	—
25 ,,	—	30	176	—
22 Dec.	—	—	14	—
21 Jan.	592	—	—	—
26 Feb.	—	13	—	—
11 Mar.	—	—	—	29
5 Apr.	446	—	—	—

(II) *Macrosiphum solanifolii* Ashm. This species is generally supposed to hibernate on roses, but, as in North Wales (Thomas & Jacob, 1943), no evidence was found in support of this view. However, in the winter of 1942–3 numerous apterous viviparous females overwintered on *Rumex obtusifolius* L., in the King's College grounds and elsewhere. In the spring this species was not uncommon on strawberries, which have been shown to be an important winter host in North Wales (Thomas & Jacob, 1943).

III) *Aphis rhamni* B.d.F. The distribution of *Rhamnus catharticus*, the winter host of this species, is shown in Fig. 1. No eggs were found during the winter 1942–3, and no colonies were seen on this host plant in the spring of 1943.

(IV) *Myzus pseudosolani* Theob. This species hibernated as apterous viviparous females on foxgloves (*Digitalis purpurea* L.) in 1942–3, particularly in the western part of the Province. Neither sexuales nor eggs were found on this host. Heinze & Profft (1940) have found overwintering eggs on *Myosotis alpina* (the author is not clear what exactly is meant by this species; if it is *Myosotis alpina* Lapeyr., then it is a synonym of *Myosotis sylvatica* Hoffm.), and *Capsella bursa-pastoris* Med. in Germany.

The winter 1943–4 was very similar to that of 1942–3, and *M. persicae*, *M. solanifolii*, and *M. pseudosolani*, all overwintered as apterous viviparous females in considerable numbers. As in the previous year, eggs of *A. rhamni* could not be found.

WEATHER DATA; WITH SPECIAL REFERENCE TO THE SPRING MIGRATION

Table 4 shows the number of days during May, June and July, from 1929 to 1943, on which weather conditions were suitable for the flight of *M. persicae* at Newton Rigg and Cockle Park (near Penrith and near Morpeth respectively, see Fig. 1).

TABLE 4. *Summary of the number of days suitable for the flight of* Myzus persicae, *at Newton Rigg and Cockle Park, during May, June and July, from* 1929 *to* 1943

Year	Month	Total no. of days with wind velocity 0–4 m.p.h.			
		Temp. 70° F.		Temp. 60–70° F.	
		Newton Rigg	Cockle Park	Newton Rigg	Cockle Park
1929–34	May	—	1	—	7
	June	—	3	—	8
	July		4		3
1935	May	0	0	5	3
	June	2	2	1	4
	July	4	7	0	4
1936	May	2	0	5	2
	June	2	2	1	2
	July	0	0	2	0
1937	May	0	0	3	3
	June	0	1	2	2
	July	3	2	2	4
1938	May	0	0	1	0
	June	1	0	2	4
	July	0	0	1	4
1939	May	3*	0	10	1
	June	7*	1	5	2
	July	0	2	6	1
1940	May	2	0	7	3
	June	12	8	2	2
	July	2	0	2	3
1941	May	2	0	3	0
	June	7	0	8	3
	July	8	0	0	1
1942	May	0	0	6	2
	June	7	0	7	0
	July	2	1	5	3
1943	May	2	1	4	3
	June	4	0	2	3
	July	8	2	2	5
Totals	May	11	2	44	24
	June	42	17	30	30
	July	27	18	20	28
No. of years		9	15	9	15

* These were ten successive days in May and June.

These two stations have been chosen mainly because their records were readily available and also because they are fairly representative of the cool moist and cool dry climates of the north-west and the north-east respectively. Otherwise, the choice is purely arbitrary. Newton Rigg has the disadvantage of being situated in a comparatively dry part of Cumberland, with the result that this station is not typical of the north-west as a whole. However, it would not be possible to illustrate the trends of weather adequately without the use of a large number of stations. Each station is fairly close to established seed potato growing districts.

The arbitrary standards adopted for Table 4, are based on those postulated by Thomas & Vevai (1940), whose results make it clear that aphis flight is important outside the limits set by Davies (1935, 1936). Thomas & Vevai suggest the following arbitrary standards for aphides in general:

(*a*) A temperature above 70° F.
(*b*) A relative humidity below 80 %.
(*c*) A wind velocity below 5 m.p.h.
(*d*) A big difference in maximum and minimum temperatures. (More than 12° F.)

The arbitrary standards on which Table 4 is based are: temperatures of over 70° F. and from 60 to 70° F.; relative humidity of 75 % and less; wind velocities calm—4 m.p.h. (Beaufort numbers 0 & 1). As no relative humidity data are available at Cockle Park, the standard adopted was to count only days with more than 5 hr. sunshine.

In spite of obvious limitations, Table 4 provides an interesting comparison between the two stations. It may also be compared with the more detailed data for Aber, Caernarvonshire, given by Thomas & Vevai (1940); and also with that published by Loughnane (1940), in which the arbitrary standards: wind force 2 (Beaufort), temperature over 50° F., relative humidity less than 70 %, were chosen. During 1938 and 1939 in Eire, the conditions for migration at the inland station of Birr were more favourable than at any of the eastern or western stations. Profft's (1939) data appear to be in keeping with those of Thomas & Vevai (1940).

1935 and 1936 in contrast, and 1937 and 1938 in common with Aber do not appear to have been favourable years for aphis migration at either of these two northern stations. At Newton Rigg, 1939 appears to have been a good year, in common with Aber, but, in very marked contrast to Cockle Park; this contrast with Cockle Park is also shown in 1941 and 1942. Conditions appear to have been favourable at each centre in 1940 which seems to have been the only good year at Cockle Park, from 1929 to 1943.

Winter data at each of these stations suggest that really severe conditions are the exception rather than the rule, and it seems safe to suggest that in the period 1928–43, *M. persicae* could have overwintered successfully on savoys in all but 1935–6, 1939–40, 1940–1, and 1941–2. Of these, 1939–40 was exceptionally severe, and it is possible that in each of the other years *M. persicae* might have overwintered successfully on savoys growing in sheltered situations. This question is important in relation to Table 4, since it is obvious that spring conditions are of diminished importance if the spring population of aphides is small.

Discussion

The contents of Tables 1 and 2 require little explanation. As would be expected, the initial infestations of *M. persicae* were greater in 1943 than in 1942; this is also true of *M. solanifolii*. In 1942 the slow development of the peak population of each species (late July in the west, late August in the east), was probably correlated with the low initial infestations. In 1943 populations did not develop as quickly as would have been expected from a study of the overwintering data and the fairly general infestation in June. This is considered to be a result of the cool wet summer experienced in 1943. The highest populations of *M. persicae* and *M. solanifolii* were recorded in close proximity to urban areas, which is clearly an indication of the influence of market gardens, kitchen gardens, and allotments, on the incidence of *M. persicae* in particular. This is in keeping with the observations made by Davies (1939) during his Scottish survey. The highest populations have been noted in the following districts: Tweedside, Lower Tyne, Lower and Middle Tees. Otherwise, few high populations were recorded.

The case of *A. rhamni* is somewhat specialized. In each of the two years this species showed a very marked correlation with the distribution of its winter host. Apart from the counts made, evidence was obtained that in 1942, infestations were very heavy in south Durham and the Kendal area; this is supported by its widespread dispersal in the four counties. In 1943, *A. rhamni* was very rare and with one exception occurred only in close proximity to its winter host (Fig. 1).

M. pseudosolani was not noted in great numbers at any centre.

Of other species, the only notable observation was 51 *Myzus ornatus* Laing/100 leaves, at Ambleside, 19 Aug. 1943.

Parasitized specimens were not numerous in any of the counts, but parasites probably helped to account for the disappearance of *A. rhamni* in 1942. Certain predators were very numerous in 1943, *Coccinella 7-punctata* L. occurred in very great numbers in the Tees area, and after eliminating the aphides resorted to cannibalism. *Syrphus balteatus* De Geer was also very common.

Conclusions

It is not possible in the light of only two years' observations to form any general conclusion respecting the importance of this survey to the problem of seed-potato production in the Northern Province, but it may be suggested that, provided districts are well isolated from the sources of spring infestation already mentioned, there seem to be large areas well suited to the maintenance of healthy seed potatoes, particularly in Cumberland and Westmorland, and the more hilly western parts of Northumberland and Durham. Northumberland is favoured by the comparative infrequency of really good migration conditions in June (Table 4), the western counties

are probably favoured by the comparatively high relative humidity of the hilly regions. In this connexion it should be remembered that Newton Rigg is typical of only a limited district.

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Beet eelworm (*Heterodera schachtii* Schm.) in East Anglia, 1934–1943

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(With 2 Text-figures)

Observations made on the beet eelworm (*Heterodera schachtii* Schm.) between 1934 and 1943 are described. This eelworm confines its attacks to crop plants of the families Chenopodiaceae and Cruciferae. Cysts similar to those of the beet eelworm have been recorded on many weeds. Records of beet eelworm on weeds of the above families are probably authentic, but those of other families require confirmation. In the field, the four so-called strains of *Heterodera schachtii* (viz. the beet, oat, pea and potato strains) behave as distinct species. Some evidence is given for the possible existence of a cruciferous strain.

Attempts were made to ascertain the distribution of beet eelworm in East Anglia. A general search was conducted throughout the area and special attention paid to beet-sugar factories, beet-loading sites, sewage farms, and to certain important beet-growing areas located in the Isle of Ely and neighbouring counties. Throughout the search, diagnosis was based on the finding of lemon-shaped cysts on the roots of sugar beet, red beet or mangolds. Details of our present knowledge of the distribution of beet eelworm are given. It appears to have been present in Britain for a considerable time but is not yet widely disseminated.

The spread of beet eelworm is considered in detail, and it is concluded that the establishment of the beet-sugar industry has led to a considerable increase in the rate of spread.

Measures for the control of beet eelworm are described. In the early days of the industry, beet was grown without regard to rotation, thus providing conditions similar to those existing in Germany before 1870 when 'beet-sickness' was becoming a serious problem. The first control measure was the introduction of a clause into contracts forbidding the growing of beet after beet or mangolds. More effective measures, which were adopted in 1939, are described. In 1943, the Sugar-Beet Eelworm Order was issued enforcing these measures over a wide area and extending them to cover all susceptible crops.

Effective control measures may result in a reduced rate of spread of the beet eelworm but cannot eliminate it.

Introduction

In 1928 at Hambrook near Bristol, Gloucester, Staniland and Walton found mangolds attacked by an eelworm of the genus *Heterodera*. Dr M. J. Triffit showed experimentally that this eelworm, which had lemon-shaped cysts, would also attack various other crop plants and weeds belonging mainly to the Chenopodiaceae and Cruciferae (Triffit, 1929). In the light of experience, there is little doubt that the eelworm concerned was the sugar-beet root eelworm *Heterodera schachtii* Schm., although at the time of discovery its identity was not clear. This record is, therefore, the first of the occurrence of beet eelworm in Great Britain. A similar discovery on mangolds was made by Mr H. W. Thompson (*in litt.*) at Llanishen near Cardiff, Glamorgan, the first and only record of beet eelworm in Wales.

In 1934, during a tour of continental beet-growing areas (Petherbridge & Stirrup, 1935), one of the writers was impressed by the widespread occurrence of beet eelworm and the serious losses it had caused. On returning, a search for this pest was at once started in East Anglia, and in November of the same year beet eelworm was found attacking sugar beet at Chatteris in the Isle of Ely (Petherbridge, 1939).

The present paper is concerned with the position revealed by a careful search up to December 1943. It also deals with observations made during this period and with control measures recommended from time to time.

Observations on the bionomics of the beet eelworm

Host range

Observations on the host range of the beet eelworm made from pot tests, trial plots and the examination of growing crops, showed that beet eelworm may attack the following crop plants:

Chenopodiaceae	Cruciferae	
Sugar beet	Turnips	Cauliflower
Red beet	Swedes	Rape or cole seed
Mangolds	Cabbage	Mustard
Spinach	Kale	Cress
	Brussels sprouts	Radish
	Broccoli	

A careful search was made of the roots of wheat, barley, oats, potatoes, chicory and peas, but on these no cysts were found. On other occasions, these crops, and also celery and carrots, have been observed growing on heavily infested land without showing signs of attack.

The above results are in general agreement with the findings of Thorne (1941) in the U.S.A., and with the experiments carried out by Triffit (1929) on the eelworm attacking mangolds near Bristol in 1928.

An attempt was made to estimate the degree to which various susceptible crops are infested. Short rows of seed were sown in the corner of a heavily infested field. At intervals plants were lifted and examined, but it soon became apparent that any accurate comparison of the degree of infestation on different hosts would be difficult to measure. However, a method was eventually obtained in which marks were given (0–5) to represent the number of cysts on the root system of each plant as estimated by eye. This method undoubtedly has limitations but gives a rough guide to the capacity of the plant to produce cysts. Table 1 gives the figures thus obtained on 13 July 1937.

TABLE 1. *The degree of infestation of crop plants susceptible to beet eelworm*

Host	Family	Total marks for 15 plants
Mangold	Chenopodiaceae	66
Red beet		59
Sugar beet		54
Spinach		41
Swede	Cruciferae	46
Turnip		40
Cress		34
Cabbage		31
Brussels sprout		30
Mustard		30
Kale		26
Broccoli		25
Cauliflower		24
1000-head kale		22
Radish		19
Oat	Gramineae	1 cyst*
Pea	Leguminosae	0

* Probably the oat eelworm (*Heterodera major* O. Schm.).

It will be noted that one cyst was found on oats. It is impossible to say whether this infestation was due to beet eelworm or whether oat eelworm was also present in the field, but the latter had never been found on the farm before.

Cysts of the genus *Heterodera* similar to those found on beet have been recorded on many weeds. Records for weeds of the families Chenopodiaceae and Cruciferae are probably authentic in most cases, but some records for weeds of other families require critical confirmation. In East Anglia cysts of the beet type have been found on the following weeds in fields infested with beet eelworm (Table 2).

In one field examined, lemon-shaped cysts were found on the roots of *Galeopsis speciosa* and not on the roots of sugar beet even when both were growing with their root systems interlocked. In the same field, cysts were also found on *Stellaria media* and *Galium aparine*, while in another part of the field sugar beet was itself attacked. On other occasions *Galeopsis speciosa* has been found attacked on land where no beet eelworm has been detected. Previously we had regarded this weed as a host of beet eelworm, which indeed it may still be. It is evident that some of the weeds listed may not be the hosts of beet eelworm but of other eelworms with cysts of the beet type.

TABLE 2. *Weeds found bearing cysts of the beet type on land infested with beet eelworm*

Caryophyllaceae	
Chickweed	*Stellaria media*
Chenopodiaceae	
Fat hen	*Chenopodium album*
Sowbane	*C. hybridum*
Common orache	*Atriplex hastata*
Cruciferae	
Charlock	*Brassica sinapis*
Hedge mustard	*Sisymbrium officinale*
Shepherd's purse	*Capsella bursa-pastoris*
Wild radish	*Raphanus raphanistrum*
Treacle mustard	*Erysimum cheiranthoides*
Labiatae	
Hemp nettle	*Galeopsis speciosa*
Polygonaceae	
Broad leaved dock	*Rumex obtusifolius*
Knotweed	*Polygonum aviculare*
Rubiaceae	
Cleavers	*Galium aparine*
Solanaceae	
Black nightshade	*Solanum nigrum*
Urticaceae	
Small nettle	*Urtica urens*

Possible existence of a cruciferous strain

Cysts of the beet type were found on swede seed plants at Terrington St Clement's, Norfolk. Pot tests with soil from the field concerned failed to give positive results with sugar beet. Later, the field was visited when growing sugar beet for seed and kohl-rabi. No cysts could be found on the beet seed plants though present on kohl-rabi and self-sown swedes. Cysts of the beet type were also found on Brassicae but not on red beet on allotments near Peterborough. Soil samples revealed a fairly high cyst and egg content for some allotments. These experiments suggest the existence of a species of eelworm distinct from the beet eelworm, with lemon-shaped cysts but attacking Brassicae and not beet and its allies. Cysts of the genus *Heterodera* have been reported on Brassicae from several Advisory Provinces (*in litt.*). Beet eelworm may be responsible in some cases but, in the absence of further information, the records remain *sub judice*.

The specific status of the beet eelworm

Most early workers included in the species *Heterodera schachtii* Schm. at least four biologically distinct strains, viz. the beet, oat, pea and potato strains. Franklyn (1940) has recently given additional reasons for according specific status to all four. Our own observations support this step so far as the sugar beet and potato eelworms are concerned. In the field they behave as distinct species, beet eelworm confining its attacks to the susceptible crops already mentioned and potato-root eelworm to potatoes and tomatoes. O'Brien & Prentice (1931) attempted the transference of potato eelworm to sugar beet. They failed in the first year, but in later years several beets were noted suffering slightly from eelworm attack. The shape of the resultant cysts was not stated. Presumably they were of the potato type, although the authors conclude that the beet and potato eelworms are not distinct species because the morphological features by which they are distinguished disappear when the potato eelworm is induced to attack sugar beet. In this connexion both Zimmermann (1927) and Goffart (1928) found that potato eelworm could be made to attack sugar beet but that the resulting cysts were lemon-shaped. More recently Robertson (*in litt.*) has obtained results similar to those of O'Brien & Prentice. However, in this case the cysts produced by potato eelworm on sugar beet are definitely of the potato type though subnormal in size. It seems unlikely that potato eelworm would change its characteristic cyst shape when attacking abnormal host plants.

Field observations made on oat and pea eelworms, though fewer than in the case of sugar beet and potato eelworms, support the view that these also are distinct species. During experiments on the host range of beet eelworm, pots of soil containing cysts of the oat eelworm were sown with oats, sugar beet, swedes, turnips and kale. Only the oats were attacked.

The search for beet eelworm in East Anglia

Diagnosis

Because of the possible existence of other root eelworms with cysts of the beet type attacking Brassicae and weeds, it is clear that only a limited number of host plants can be used for the diagnosis of beet eelworm. During the search for beet eelworm in East Anglia, diagnosis has been based entirely on the occurrence of lemon-shaped cysts on the rootlets of sugar beet, red beet or mangolds. Where eelworm cysts have been found only on weeds or cruciferous crops, the occurrence has been noted and the field visited again when growing one of the diagnostic crops.

In most cases diagnosis has been made in the field on growing crops; in a few it has been made on clamped mangolds or red beet; and, with one exception, pot tests have only been used in the examination of wind-blown soil or waste soil from beet-loading sites. Where diagnosis has been made on roots clamped in a field different from the one in which they were grown, then both fields have been regarded as infested, for cysts and infested soil inevitably fall from the roots to infest the clamp site.

Beet-sugar factories

In 1934 the examination of soil washings from sugar beet at four factories revealed the presence of large numbers of lemon-shaped cysts. Similar observations were made at two factories in Yorkshire by Mr H. W. Thompson (*in litt.*), although at one of those most cysts were of the potato type. In 1935, samples of soil were taken at a number of factories from beets showing a marked development of lateral roots. 177 samples were examined for eelworm by floating them out in water, and of these 70 contained viable lemon-shaped cysts. Many of these samples were traced to the fields from which they came. Here lemon-shaped cysts were found on wheat, barley and wild oats (no oats being grown), but not on beet. Pot tests also gave positive results for oats but not for sugar beet or peas. This suggests that the oat-root eelworm (*H. major*) is widely distributed in the eastern counties, but no case of serious damage has yet been encountered.

The direct examination of incoming beets was attempted at several factories over a period of 5 years, but few cases of beet eelworm infestation were traced by this means. This method has limitations, for many loads arrive in a condition unfit for examination, and only a small proportion of the total number entering a factory can be scrutinized.

It is therefore concluded (1) that lemon-shaped cysts are present in many fields on which sugar beet is grown but that very few are those of the beet eelworm, and (2) infested beets form a very small proportion of the total tonnage received at the factories.

Beet-loading sites

The examination of waste soil from beet-loading sites, undertaken during the winters from 1940 to 1943, gave results which led to the discovery of a number of new centres of eelworm infestation. This waste soil, when it comes from infested fields, is a concentrated source of eelworm cysts. A load of sugar beet of about 1 ton contains some 3000 separate beets and leaves behind in the bottom of the cart after unloading, a deposit of waste soil weighing from $\frac{1}{2}$–1 cwt. If the beets have come from slightly infested land and an average of one cyst falls from each root, then the waste soil would contain 3000 cysts. Since the cysts have been freshly produced, the average egg content might be 300 eggs or more per cyst. The total egg content of the waste soil

would therefore be about 1,000,600, giving a concentration of the order of 30 eggs/g.—probably sufficient to give rise to symptoms of 'beet-sickness' and much higher than the concentration in the field from which the soil originated. This may be readily tested by shaking infested roots and examining the resultant soil for cysts. Further evidence has also been obtained in the field. A farmer, wishing to make a single large field from two small fields, filled in the intervening ditches and levelled the land with waste soil from beet carting. As a result, the sites of the ditches and parts of a newly ploughed grass field were 'beet-sick'.

Large quantities of waste soil accumulate at beet-loading sites where carts are emptied and roots are dumped to await transport to the beet factories. The soil is readily distinguished by its texture and the large quantities of beet debris which it contains. Loading sites may be roughly classified as follows:

(1) Field loading sites—waste soil representative of the whole field.

(2) Farm loading sites—waste soil representative of several fields on the farm.

(3) River loading sites—waste soil representative of fields on the farm or farms using the site.

(4) Railway sidings—waste soil representative of the farms using the sidings.

Occasionally dumps of waste soil may be encountered which have been accumulating for several years. At railway sidings, farmers are not generally allowed to clean out their carts or lorries and leave waste soil behind. Nevertheless, considerable quantities of waste soil spill about and are removed from time to time by the platelayers. These are deposited in old pits or at some convenient spot along an embankment. One case is known where waste soil has rolled down on to arable land. A private railway, important for beet traffic since it serves a beet-sugar factory, appears to have no regulations about waste soil, and very large quantities are left at the sidings every year.

Representative samples of waste soil were taken from loading sites in selected areas and submitted to pot tests in the laboratory. Rough counts of cysts and eggs were also made as an additional guide. The results obtained are set out in Table 3.

With one exception, the source of eelworm at all the infested sites has now been traced. Apart from the sites on the River Yare serving an infested sewage farm, all the sites examined lie in the black fen areas of East Anglia, but the results do not give a general picture of the proportion of loading sites infested in these areas because they were not chosen at random.

Sewage farms

In East Anglia twenty-three sewage farms have been examined at various times. Of the fourteen growing crops susceptible to beet eelworm, seven were infested. Elsewhere, three more sewage farms are known to be infested, bringing the total to ten.

TABLE 3. *Results of the examination of waste soil at beet-loading sites*

Type of site	Locality or river	No. examined	No. infested
Roadside loading sites	Benwick, Isle of Ely	15	0
	Crowland, Holland, Lincs.	15	0
	Ely, Isle of Ely	1	0
	Lakenheath, West Suffolk	4	1
	Waterbeach, Cambridge	14	0
	Wimblington, Isle of Ely	7	0
River loading sites	Cam	18	0
	Lark	11	1
	Little Ouse	10	4
	Great Ouse	15	3
	Wissey	1	1
	Yare	1	1
Railway sidings	A private line serving Methwold and Feltwell Fens	20	5
	Main line companies	12	3
	Total	144	19

Susceptible crops figure largely in the cropping of some sewage farms (see Table 4):

TABLE 4. *The cropping of sewage farms*

Farm no.	Cropping
1	Mangolds and Brassicae alternately
2	Frequent crops of sugar beet
3	Mangolds every year
4	Mangolds every year until 1937, now given over to allotments
5	Frequent crops of mangolds and sugar beet
6	Frequent crops of sugar beet; mangolds and Brassicae also grown
7	Mangolds and occasionally Brassicae
8	Cropped for 40 years with roots, mainly mangolds

Such farms form ideal centres for the propagation of beet eelworm and are to be found scattered throughout the country. At some infested farms mangolds were severely attacked, and it appeared that beet eelworm had been present for a considerable time.

As beet eelworm was found in so large a proportion of the sewage farms examined, it seems likely that they are particularly liable to infestation. This may possibly be due to the washing of vegetables in kitchen sinks so that cysts and waste soil find their way into sewage. However, crude sewage is usually submitted to various treatments before being applied to land either as effluent or sludge, and it is not clear that eelworms would survive, except perhaps in the first coarse screenings. The treatments vary considerably, and in assessing the chance of eelworm

survival each sewage farm would have to be separately considered. In this connexion, it should be mentioned that certain sewage farms which have grown susceptible crops frequently over long periods appear to be free from beet eelworm and continue to grow excellent crops.

There appears to be some danger of the spread of eelworm by the sale of sewage sludge as fertilizer, especially from infested farms; for here, even if the sludge is free from eelworm at the time of production, there is a risk of contamination with infested soil during loading and carting. The greatest danger, however, is probably from the sale of infested mangolds and Brassicae for feeding.

Sewage farms are rare in the fens and do not appear to have played any part in the spread of beet eelworm there.

The Fens

In 1937 an intensive survey was started in the peat fens of the Isle of Ely and neighbouring counties, this area being singled out for special attention because it is one of the most important beet-growing areas in the country. At first the survey was limited to those localities where beet eelworm had previously been found as a result of a general search between 1934 and 1937. The object was to discover the number of fields affected and the extent of spread to neighbouring land. By 1939 it was clear that beet eelworm was more prevalent than had previously been supposed and, from then onwards, the scope of the survey was widened to cover other localities where frequent cropping with sugar beet had been practised. Every effort was made to trace and survey fields, farms and localities where it seemed likely that beet eelworm might exist. Some information on this point was collected during the normal activities of the Advisory Centre, but most was forthcoming from the numerous contacts with farmers, factory fieldmen, and staffs of County War Agricultural Executive Committees, during the survey itself.

With the aid of Ordnance Survey Maps (6 in. to 1 mile), which show field boundaries, each area under survey was covered field by field. In those fields growing sugar beet, red beet or mangolds, plants were lifted here and there and carefully examined for the presence of eelworm cysts. Special attention was given to any obviously bad patches in the crop and also to certain parts of fields which experience had shown to be the most likely places for an infestation to be found. These were:

(1) Land around gateways.
(2) Land alongside unfenced roads.
(3) Headlands.
(4) Sides of fields.
(5) Land near farm buildings.
(6) Sites of beet heaps and of red beet and mangold clamps.

Gateways are foci of farm movements. For this reason land near gateways is particularly exposed to the risk of infestation. The same is also true, to a lesser extent, of headlands. Land alongside unfenced roadways is liable to infestation by soil or infested beet falling from carts or implements, and if the roadways become impassable in winter, carting may occur along the edge of the field itself. The edges of fields grow susceptible crops more frequently than the remainder, and land near stockyards may grow frequent crops of mangolds or Brassicae for feeding as a matter of convenience. The same applies to fields near hard roads which make carting easy. Disused gateways, roadways or stockyards, sometimes shown on maps, are worth investigation. The sites of clamps, particularly those of sugar beet, mangolds or red beet, are of interest because of the waste soil left behind after their removal. On some farms, sugar beet is piled or roughly clamped on easily accessible land to await transport. Examination of susceptible crops growing on the clamp sites gives an indication of the condition of the farm.

The survey was carried out each year during the months of July, August, September and October. Before the end of June, most of the cysts are immature and difficult to detect. At the end of October, much beet has been lifted and weather is often adverse. Muddy soil also increases the danger of spreading eelworm. This danger is always present in wet weather but can be reduced to a minimum by cleaning boots and trowels after leaving infested land, and by working known infested areas on different days from areas where eelworm is apparently absent.

Results of the search

Table 5 gives a list of localities where beet eelworm has been found. Records made outside East Anglia are included for completeness.

Fig. 1 shows our present knowledge of the distribution of beet eelworm throughout the country, while Fig. 2 gives more details for the Fens. In the latter, the smallest areas where beet eelworm is known to exist are single fields, while the larger areas include both infested and uninfested fields. On the whole, the proportion of infested to uninfested fields is greatest in areas such as the Somersham Road district of Chatteris in the Isle of Ely where beet eelworm appears to have been present longest. The total area to which the survey had been extended by 1943 was 130 sq. miles; this is about a third of the area enclosed by the continuous black line in Fig. 2.

Detailed results of the search for beet eelworm, in terms of the number and area of infested fields, are given in Table 6. The field has been used as the unit infested area because field boundaries form the first temporary barriers to eelworm spread. Field areas have been taken from the Ordnance Survey

TABLE 5. *Records of centres of beet eelworm infestation, up to 31 Dec. 1943*

Year	County	Locality
1928	Gloucester	Hambrook, Bristol (Staniland & Walton)
1932	Glamorgan	Llanishen, Cardiff (Thompson)
1934	Isle of Ely	Somersham Rd, Chatteris (Pether-bridge)
1935	Isle of Ely	Doddington Rd, Chatteris
		Hockwold-cum-Wilton
		Larman's Fen, Southery
1936	Lincoln	Graizelound, Haxey (Roebuck)
	Norfolk	Great West Fen, Hilgay
		Harling Road, East Harling
		Ten Mile Bank, Hilgay
		*Whittlingham, Norwich
		†Wissington, Stoke Ferry
1937	Bedford	Langford
	Norfolk	Downham West, Downham Market
		Fordham Fen, Hilgay
1938	Cambridge	East Fen Drove, Soham
	Leicester	*Loughborough (Roebuck)
	Lincoln	Market Rasen (Roebuck)
1939	Derby	*Eggington and Etwall Commons (Roebuck)
	Huntingdon	Somersham
	Isle of Ely	†Adelaide, Ely
		Sutton
		Sutton Gault
		Warboys Road, Chatteris
	Norfolk	'Highland', Hilgay
		Southery
1941	Isle of Ely	New Road, Chatteris
	Norfolk	Brandon Creek
		‡King's Lynn
1942	Cambridge	Fodder Fen, Soham
	Huntingdon	Gaunt Fen, Warboys (Nr Chatteris)
		Ramsey
		Ramsey Heights
		Ramsey St Mary's
	Isle of Ely	§Ely
		Little Downham
		Manea
	Norfolk	Bellmaco, Hilgay
		Jacob's Farm, Feltwell Fen
		Methwold Hithe
		Roxham Fen, Hilgay
		Shrubhill Farm, Feltwell Fen
	West Suffolk	*Haverhill
		Mildenhall Fen
1943	Cambridge	Isleham
		Great Hasse Drove, Soham
		Northfield, Soham
	Huntingdon	Ugg Mere
		Yaxley
	Hertford	*Hitchin
	Isle of Ely	*Northey Gravel, Peterborough
		Littleport
	Norfolk	Feltwell Anchor
		Feltwell Common
		Poppylot, Southery
		Toodleham's Farm, Southery
	Northampton	*Higham Ferrers
		*Rushden
	Shropshire	*Admaston, Wellington (Newton)
	Soke of Peterborough	Eye Green, Eye
		Hurn Farm, Eye
	West Suffolk	Lakenheath
		*West Stow, Bury St Edmunds
	East Riding, Yorks.	South Cave (Thompson)

* Infested sewage farms.
† The waste soil dumps of two beet-sugar factories situated in these areas, were subsequently found to be infested.
‡ Waste soil dump at beet-sugar factory. § Includes the City of Ely rubbish dump.

TABLE 6. *The beet eelworm position in East Anglia up to December* 1943

	1934–6		1937–40		1941		1942		1943		Totals		Heavily infested land		Area surveyed by 1943 in sq. miles
County	Fields	Acres	Fields	Acres	Fields	Acres	Fields	Acres	Fields	Acres	Fields	Acres	Fields	Acres	
Bedfordshire	—	—	1	5	—	—	—	—	—	—	1	5	—	—	—
Cambridgeshire	—	—	2	37	—	—	3	54	27	239	32	330	8	46	10
Huntingdonshire	—	—	5	44	5	55	5	51	12	163	27	313	—	—	10
Hertfordshire	—	—	—	—	—	—	—	—	1	5	1	5	—	—	—
Isle of Ely	25	198	111	951	34	351	72	684	41	297	283	2481	38	310	55
Norfolk	16	210	66	575	19	197	35	374	50	637	186	1993	38	356	45
Northamptonshire	—	—	—	—	—	—	—	—	3	8	3	8	1	2	—
Soke of Peterborough	—	—	—	—	—	—	—	—	7	111	7	111	—	—	4
West Suffolk	—	—	—	—	—	—	5	40	11	105	16	145	4	26	6
Totals	41	408	185	1612	58	603	120	1203	152	1565	556	5391	89	740	130
Heavily infested land	18	139	29	217	11	103	13	138	18	143	89	740	—	—	—
Area surveyed in sq. miles	None		6–40		50		100		130		130		—		—

Based upon the Ordnance Survey Map, with the sanction of the Controller of H.M. Stationery Office.

Fig. 1.

Fig. 2. ■ Localities in which beet eelworm is known to exist. —— Boundary of the *Infected Area* defined by the Sugar-Beet Eelworm Order of 1943. - - - - Proposed extensions of the *Infected Area* boundary.

maps of the 25 in. to 1 mile series, except in a few instances where they have been estimated by eye from the 6 in. to 1 mile series.

For control purposes it is necessary to distinguish between heavily infested fields and those less seriously affected. Fields placed in the heavily infested category are those in which eelworm cysts were widespread and abundant upon the roots, and where there was an appreciable loss of yield due to eelworm attack (Table 6, 'Heavily infested land'). On an average, one field in every six or seven detected was placed in this category.

During the survey, beet eelworm was found on most types of black fen soil varying in texture from light to heavy and including mixtures of fen peats with wide varieties of mineral materials. Eelworm appears to be absent from those areas of fen which are markedly acid, probably because they are unsuited to the growing of sugar beet. Outside the Fens, beet eelworm has been found on sand, light loam, medium loam and alluvium, but not on heavy soil where sugar beet is grown less frequently.

All grades of infested fields have been encountered, from those where only a few cysts could be

found in an isolated spot and where the infestation appeared to be in an early stage, to those where cysts could be found throughout the field and the infestation appeared to be of long standing. Sometimes the effects of infested waste soil emptied from carts during beet carting could be distinguished by the presence of a number of extremely small 'sick' patches. In a few fields, a single 'sick' patch of this type was the only infestation discovered, cysts being extremely abundant in the patch and apparently absent from the remainder of the field. Most of the extensively infested fields discovered were 'beet-sick'. Those which were not, had probably been 'beet-sick' in the past.

In the areas surveyed, all extensively infested fields growing diagnostic crops were detected, as also were most of the moderately infested fields, but probably some slightly infested fields were missed.

Beet eelworm was more frequently encountered on allotments, smallholdings and small farms than on large farms. This appears to be due to the suitability of the sugar-beet crop to the requirements of the small grower and consequently to the greater frequency with which it is grown. In addition, plots of mangolds and Brassicae are grown for stock, so that with a limited amount of land available, it is often difficult to provide a sufficient period of rest between each susceptible crop.

On many farms, certain fields particularly suited to sugar beet or conveniently situated near beet-sugar factories, railway sidings, waterways or hard roads, are frequently cropped with sugar beet. It is in such fields that the first signs of eelworm attack are usually apparent. Another practice met with on mainly grassland farms is the setting aside of certain fields ('root fields') to grow mangolds and Brassicae for stock. The first two records of beet eelworm in Great Britain (Table 5) appear to have been made in fields of this type, and more were encountered during the survey. Such fields occur throughout the country, and it is possible that some have been infested for a considerable time.

In conclusion, it may be added that the survey has provided evidence (if any more were needed), that the principal cause of 'beet-sickness' is frequent cropping with plants susceptible to beet eelworm attack.

Eelworm spread

The establishment and progress of beet eelworm infestations

To establish an infestation on clean land the introduction of living eelworms and the presence of suitable host plants are necessary. The initial introduction is usually by means of cysts. Free eelworms may be introduced into infested soil, but cysts are of greater importance, as the eggs which they contain are capable of retaining their viability over long periods. For this reason it is not essential that host plants should be present in the year of introduction. The chances of establishment will be greater if cysts are freshly produced (i.e. full of eggs with maximum viability), and if they arrive in numbers. It has already been shown that waste soil from beet carting may be especially potent in both respects. Beet eelworm, with its wide range of host plants among crops and weeds, has better chances of survival than other eelworms with more limited host ranges. In districts where sugar beet is grown at regular intervals in a four-course or shorter rotation, the chance of establishing an infestation from relatively few cysts amounts almost to a certainty.

The spread of eelworm due to the movements of larvae in the soil is small, amounting at the most to a few yards per season (Baunacke, 1922), but that due to the intervention of man in transporting infested soil containing cysts is so great as to overcome all but the most impassable barriers. The rate of spread depends, in the first place, on the area already infested and the degree of infestation, as these two factors together determine the number of opportunities afforded for the dissemination of cysts. Field boundaries offer the first resistance to eelworm spread, but farm boundaries, roads, railways, river and large tracts of permanent grass form more efficient barriers. As more and more land becomes infested and the degree of infestation increases, so also does the rate of spread; the final stages leading to widespread infestation are likely to be surprisingly rapid. This appears to have been the history of beet eelworm on the continent and in the U.S.A. Thorne (1941), describing the position in the United States, says: 'The sugar-beet nematode has been known to be present in the U.S.A. for about 35 years. During this time, new infestations have developed in such numbers and the old infested areas have so increased in size, that in many sections the control of this pest now ranks as one of the major problems confronting the beet-sugar industry. These widespread infestations indicate that eventually every important beet-growing section in the Western States will probably become infested.'

The history of potato root eelworm in Great Britain has followed much the same course and the end-point—widespread infestation—is probably not far off.

The dissemination of eelworm cysts

The means by which the transport of eelworm cysts, or soil containing cysts, may occur have been reviewed by many workers, and are summarized below:

(1) *General farm activities.* Soil adhering to the boots of farm workers and others, to the feet of farm animals and birds, to implements, tractors, carts, lorries, produce, and also to sacks and many other articles.

(2) *Tubers, transplants, etc.* Soil adhering to seed potatoes and other tubers, and also to bulbs, corms, stecklings, and all kinds of transplants.

(3) *Seeds.* Small lumps of soil contained in seed, especially in the large kinds such as the seed clusters of sugar beet, red beet and mangolds.

(4) *Roots for feeding.* Mangolds, turnips, swedes and occasionally sugar beet. To these must be added potatoes for feeding, beet tops, cattle cabbage, kohlrabi and kale, and also to a lesser degree the manure produced during the process of feeding.

(5) *Sewage farms.* The sale of mangolds and Brassicae for fodder and possibly also of sewage sludge.

(6) *Waste products of beet-sugar factories.* Waste soil, beet tailings and other plant debris.

(7) *Wind.*

(8) *Water currents.*

It is clear that the means of disseminating eelworm cysts are numerous, and that whilst some are localized in their effects others might be responsible for the spread of eelworm over wide areas. Furthermore, although a measure of control might be obtained by legislation or education in some cases, in others control appears to be out of the question.

Some aspects of eelworm spread

The fact that smallholdings and small farms form less self-contained units than large farms, that they are more dependent upon the hire of implements and work done on contract, and that many smallholders also work part time on other farms or holdings, may help to explain why eelworm was more frequently encountered on smallholdings and small farms. Connexions between apparently isolated infestations were sometimes traced through workmen, the hire of implements and work done by contractors.

Various workers have drawn attention to the spread of potato-root eelworm in soil adhering to seed-potato tubers. There is, however, no record of beet eelworm occurring in Scotland or Ireland, and therefore, there seems to be little risk of the spread of beet eelworm in Scotch or Irish seed potatoes, but considerable quantities of once-grown seed potatoes are sold from farms in East Anglia.

The spread of eelworm by the sale of infested roots has been mentioned in connexion with sewage farms. Large quantities of mangolds were also sold from the Chatteris district of the Isle of Ely, where eelworm was present before sugar beet was grown. Some of these roots were sold in towns and the resultant manure probably returned to the country. Eelworm spread by the sale of infested roots and manure has probably been in progress for some time.

Various workers have commented on the occurrence of cysts in seeds. Between 1936 and 1940, 138 samples of soil from sugar-beet seed were examined at Cambridge. They were collected at various East Anglian factories and included sixty varieties grown by twenty-four seed companies, English and foreign. Lemon-shaped cysts were found in twenty-three samples, but in only four cases did the cysts contain eggs and none appeared viable. It seems possible that occasional viable cysts might occur. Practically all English-grown beet seed is now submitted to a process of drying and is dressed with organo-mercury compounds, but the effects of these treatments on beet eelworm are unknown.

In East Anglia, dust storms frequently occur during the spring in certain areas of light sandy soil and light peat fen. Investigation of blown soil on two occasions has given proof that eelworm may be spread by this means. In 1942, the Chatteris area was visited at the height of a spring gale, and many fields were observed giving off intermittent clouds of dust, while dust rose almost continuously from a few particularly bad fields. Dust storms are especially prevalent in the Methwold and Feltwell Fen areas of Norfolk. In this area beet eelworm is widely scattered (Fig. 2), but this, however, does not appear to be due to wind action. The area is served by navigable waterways and a private railway and all the infested land so far found lies along these lines of communication. Land remote from them, derelict for many years, has recently been ploughed up. It is possible that wind action may play a greater part in further eelworm spread if this land remains arable.

Thorne (1941) refers to the spread of beet eelworm by water currents and irrigation. Although an instance of the spread of potato eelworm cysts by floods has been reported in this country, the spread of beet eelworm by this means has not been observed. In the Fens, where the land is extremely flat, strong water currents occur only in the main drainage channels during winter when pumping is necessary to prevent flooding.

Sugar beet grown on infested land ultimately arrives at the beet-sugar factories. Of the eighteen factories operating in the country, eleven are known to have received infested beet as also, in all probability, have some of the remainder. Infested beets are disposed of in the process of sugar manufacture, but quantities of waste soil and plant debris remain, most of which is removed to permanent dumps. Occasionally some of these waste products, which are liable to be infested, are used for various purposes or removed from the premises. Lorries leaving the factories often contain deposits of waste soil left after unloading. This waste soil is emptied at any spot convenient to the driver without regard to the possibility of contaminating arable land. Sometimes reserves of beet are piled on farm land adjacent to the factory. This land may be infested by waste soil falling to the bases of the heaps. One field next to a factory was almost certainly infested in this way.

Lime sludge, a by-product of sugar manufacture and often applied to farm land, has occasionally been suspected of spreading beet eelworm. The sludge is sterile when first produced, and there is no risk where it is kept free from contact or admixture with waste soil. At two factories, where the lime sludge and waste soil dumps were side by side, the intervening bank was inadequate and the two had mixed. Both waste soil dumps were infested and pot tests carried out at one factory showed that the superficial lime sludge was also infested. Neither factory had sold lime sludge to farmers.

Control measures

In spite of extensive investigations carried out in many countries over a long period, no satisfactory chemical treatment capable of destroying eelworms in the soil on a field scale has yet been discovered. Of necessity, therefore, control measures are based on rotation of crops, the interval between susceptible crops being sufficient to allow the eelworm population to fall to a level at which serious damage does not occur.

Early control measures

In the early days of the beet-sugar industry in Great Britain (1925 onwards), every effort was made to extend the cultivation of the crop without regard to rotation. Moreover, the breakdown of rotations, already in progress, was fostered during the period of agricultural depression (1924–32) when sugar beet was one of the few crops producing a certain cash return. For this reason it was grown frequently in areas where the soil allowed, sometimes year after year on the same land. This state of affairs closely parallels that obtaining in Germany before 1870 when 'beet-sickness' was becoming a serious problem (Anon. 1931). Following the introduction of a clause into beet contracts in 1935 forbidding the planting of beet after beet, mangolds or certain other root crops, a common practice in some areas was to alternate beet with potatoes, or wheat or barley, although in some cases the clause was disregarded.

Before 1939, few cases of eelworm infestation were known and individual advice was given. In 1939 certain control measures were recommended to the British Sugar Corporation Ltd. and accepted. A résumé of these measures is given below:

(1) (*a*) Certain areas embracing groups of infested fields and also including a number of uninfested fields, shall be scheduled as 'sugar-beet eelworm infested areas' (Fig. 2, localities in which beet eelworm is known to exist).

(*b*) In a scheduled area sugar-beet contracts shall be handled by a single factory, so that contracts refused by one factory cannot be accepted by another.

(*c*) In each scheduled area the factory responsible shall keep a record of the cropping of every field.

(*d*) Within a scheduled area the field is regarded as the unit: where part is infested, the whole should be regarded as infested and where part grows a susceptible crop, the whole should be regarded as if it had grown a susceptible crop in that year.

(2) In uninfested fields situated within a scheduled area sugar beet shall be grown only after the land has been free from susceptible crops for TWO YEARS.

(3) In infested fields with a low eelworm population, sugar beet shall be grown only after the land has been free from susceptible crops for THREE YEARS.

(4) Infested fields with a high eelworm content ('sick fields') shall, in the first instance, grow sugar beet only after the land has been free from susceptible crops for FIVE YEARS. At the end of this period the eelworm population of the soil shall be determined and, if low, the field shall rank as an infested field with a low eelworm content (see (3) above).

At the same time it was suggested that in all beet-growing districts sugar beet should be grown only after the land had been free from susceptible crops for 2 years. Suggestions were also made concerning measures which would assist in the control of eelworm spread (see Petherbridge, 1939; and Petherbridge *et al.* 1938).

The 'Sugar-Beet Eelworm Order'

By 1941 it was apparent that beet eelworm was widely scattered throughout the country though most abundant in the Fens, and that the control measures already operating were not sufficient. It seemed more desirable than in 1939 that sugar beet and other susceptible crops should not be grown more often than once every 3 years in all beet-growing districts. However, this was hardly practicable in war time, and consequently the restriction was recommended only for a large area of peat fen soil in East Anglia, where the danger appeared greatest, and for a few additional small areas. Neither the County War Agricultural Executive Committees nor the British Sugar Corporation had the power to carry out such far-reaching control measures, and accordingly the Ministry of Agriculture and Fisheries agreed to issue an Order under the Destructive Insects and Pests Act.

The Sugat-Beet Eelworm Order (1943) (S.R. and O. 1943, No. 1136) was issued in August 1943. It enables County War Agricultural Executive Committees to enforce crop rotation on infested land anywhere and on uninfested land in certain districts specified in the Order. The rotations adopted by the County War Agricultural Executive Committees concerned are those recommended to the British Sugar Corporation Ltd. in 1939 and operated by them between 1939 and 1943 (items 2, 3 and 4 above). It has also been agreed that the field shall be taken as the unit in the case of infested land (item 1(*d*)). The Order also stipulates that no seed potatoes

raised on infested land shall be moved off such land for planting otherwise than on the farm or holding of which such land forms part, and further, confers upon the County War Agricultural Executive Committees the power of taking such other measures as may be deemed advisable to prevent the spread of beet eelworm. These provisions of the Order cover the spread of eelworm by methods (2) and (4) on page 329.

The Order is supported by a previous Order forbidding the import of sugar beet or mangold plants except under licence (Importation of Plants (Amendment) Order (1935) S.R. and O. 1935, No. 1225). In 1941 the Sugar-Beet Seed Growers' Association decided not to raise sugar-beet seed in areas where beet eelworm is known to exist (Fig. 2). In these areas also, the field staffs of the beet-sugar factories continue to keep cropping records and to exercise special care as they did before the issue of the Order.

DISCUSSION

It is not possible to decide whether beet eelworm is indigenous to Great Britain or whether it was introduced. If indigenous, its distribution seems unduly restricted for an organism with so many host plants amongst crops and weeds, unless originally confined to a few small and inaccessible localities. It cannot have been indigenous to fen soil as the Fens have not long been drained.

Isolated cases of beet eelworm infestation seem to have been scattered throughout the country before the establishment of the beet sugar industry. Farmers in the Chatteris district of the Isle of Ely, who are well acquainted with 'beet-sickness', state that mangolds were attacked before sugar beet was cultivated. Beet eelworm also appears to have been present for a considerable time in certain other areas in the Fens and in some sewage farms and 'root fields'.

At present beet eelworm is not widespread. It appears to be in the early stages of dissemination. Many uninfested fields can still be found in the Fens and elsewhere, where sugar beet or mangolds have been grown frequently and conditions appear favourable for eelworm attack. At Rothamsted Experimental Station mangolds have been grown in 'Barnfield' every year since 1876 with few exceptions, and the field is not yet infested.* Nevertheless, in East Anglia alone some 556 infested fields, comprising 5391 acres of land, are known, and the total number of infested fields is probably much greater than this. Outside East Anglia, no special search has been made and the position remains obscure.

It seems probable that the rate of spread of beet eelworm in Great Britain increased with the growth of transport by rail and later by road, and that the rate was still further increased with the development of the beet-sugar industry. Sugar beet provides an additional host plant, and the transport of this bulky crop to the factories affords many opportunities for the dissemination of eelworm cysts.

The insidious nature of the early stages of beet eelworm infestation, which pass unnoticed by the farmer, is one of the difficulties in the way of taking preventive measures before a serious situation arises. In East Anglia, only thirty-three inquiries concerning beet eelworm have been sent in to the Advisory Centre in the usual way. Most of these came through a single factory, and nearly all involved fields where beet eelworm had been present for a considerable time and had spread a good deal in the neighbourhood.

The recommendations made in 1939 and 1941 have been endorsed by subsequent results of the search for beet eelworm (Tables 5 and 6) and the 'Infected Area' defined by the Sugar-Beet Eelworm Order already requires some enlargement (Fig. 2). Control measures based on crop rotation and mainly designed to prevent serious losses of yield by keeping eelworm populations at a low level, may be expected to reduce the rate of eelworm spread to some extent but will not eliminate it entirely.

The writers wish to express their gratitude to Dr D. Price Jones, Messrs J. H. Stapley, K. P. Humphries, B. D. Wragge Morley, and others who have assisted at various times in the search for beet eelworm. Thanks are also due to the field staffs of East Anglian Beet-Sugar Factories and County War Agricultural Executive Committees for their kind co-operation.

* The mangolds in Barnfield were examined in early October 1944 and found to be fairly heavily infested with *H. schachtii*.

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Influence of earthworms on larval emergence in the potato-root eelworm, *Heterodera rostochiensis* Wollenweber

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(With 4 Text-figures)

Hatching experiments were carried out with potato-root eelworm cysts (*a*) from plant pots containing different numbers of earthworms, and (*b*) from worm casts. Cysts from these sources differed from controls in the following ways: (1) more larvae emerged, per cyst; (2) the larvae emerged sooner; (3) more cysts produced larvae; (4) the number of emerging larvae, per cyst, increased with the number of earthworms.

Examination of the contents of cysts isolated from worm casts showed that, compared with controls, 145 % more eggs hatched; it also indicated some of the limitations of the 'per-cyst' basis of expressing results.

INTRODUCTION

Isolation of intact eelworm cysts from worm casts from the field made it clear that cysts could pass through the gut of earthworms apparently unharmed, for both cysts and contents appeared to be normal. The experiments described in the present paper were designed to discover whether the viability of the cysts was affected in any way; a first series deals with cysts isolated from plant pots which had contained different numbers of earthworms, while in a second series cysts isolated from worm casts are examined.

A. SOIL EXPERIMENTS

Materials and methods

Four 10 in. plant pots were filled with thoroughly mixed soil brought into the laboratory in 1941 from a plot highly infested with eelworm, where potatoes had been repeatedly grown for a number of years. Although the soil was homogeneous, a small sample was taken from each pot and kept in a screw-top jar. In November 1942, 100 earthworms, mostly *Allolobophora longa* Ude, were added to one pot, and 60, 40 and 20 worms to the other three pots respectively. The soil was occasionally watered until February, when the worms were removed. Samples of soil were taken from each pot, and, together with the samples taken from the pots prior to the addition of earthworms, air-dried, sieved, and then left exposed to the air until the beginning of June 1943. Cysts were then separated from the soil by the flotation method and tested with potato-root excretion using the modification of the single-cyst technique of Gemmell (1940) described elsewhere (Ellenby, 1943), the individual cysts being tested in lots of 15. Altogether 135 cysts came from the various earthworm pots, and 60 from the samples of soil taken from the pots prior to the addition of the earthworms. When tested with root excretion, the age of any cyst would be not less than 3 years, often considerably more.

It was impossible to start all the series on the same day. Tests with cysts from the 100- and 20-earthworm pots and appropriate controls, were started on 1 June, while a second series, with cysts from 60- and 40-earthworm pots and their controls were commenced on 4 June; this proved fortunate. In the former, the larvae emerging were first counted on 4 June, i.e. 3 days after the beginning of the tests,

and then on the 6th day; this procedure was followed by Gemmell (1940), but as the *rate* of hatching appeared to be affected, subsequent counts were made each day until the 14th day by which time the rate of hatching had reached a low level. For the same reason, larvae from the 60- and 40-series were first counted on the third day, prior to which there is practically no hatching, and then every day. Freshly prepared root excretion was added to the cysts every 3 days.

RESULTS

Results are discussed under two heads: (1) the number of larvae produced, per cyst, and (2) the rate at which they are produced.

TABLE 1. *'Soil' experiments. Total number of larvae emerging, per cyst*

Earthworms per pot												
100			60		40		20		Control			
1	2	3	1	2	1	2	1	2	1	2	3	4
132	97	126	33	53	0	0	193	10	54	0	42	57
342	0	0	200	367	15	220	0	9	80	22	0	0
2	383	0	0	0	0	0	67	39	0	30	0	0
17	0	130	0	177	196	7	57	32	0	78	0	1
96	3	0	107	87	0	31	23	76	3	162	77	0
2	178	205	53	25	260	0	3	176	20	0	30	13
0	0	83	0	0	13	0	45	16	62	108	50	0
82	355	0	47	27	0	202	0	0	128	74	0	19
0	196	54	59	2	8	33	196	44	27	28	34	0
228	0	300	22	0	79	0	0	11	19	51	59	179
147	0	1	12	0	53	0	46	277	28	148	16	0
197	0	0	83	227	62	0	0	4	30	1	0	0
375	166	0	41	0	0	0	0	209	12	0	7	73
0	129	0	116	0	37	106	7	93	25	0	196	45
1	0	422	0	41	10	0	0	13	7	0	11	18
Mean number of larvae per cyst from all cysts												
93·8			56·9		44·4		60·6		35·4			
Mean number of larvae per cyst from productive cysts only												
158·9			85·4		83·3		71·6		51·8			
Standard error												
±16·6			±19·7		±22·0		±18·3		±13·8			

(1) *Larvae per cyst*

The data are presented in Table 1. The mean value for number of larvae per cyst increases with the number of earthworms per pot. Counting only those cysts which produce larvae, the values increase fairly steadily with increasing number of earthworms per pot; when non-productive cysts are included, the increase is less regular owing to the high and low percentages of productive cysts in the 20- and 40-earthworm pots respectively. The number of larvae per cyst, however, varies considerably within all groups; most cysts produce between 0 and 50 larvae, but values over 100 are frequent, and there are quite a number between 200 and 400. None of these most fertile cysts, incidentally, are found in the control group, most of them belonging to the 100-earthworm series. As the distribution is much skewed, it may be misleading to analyse such data as if the errors were normally distributed. Nevertheless, a number of statistical tests were carried out, excluding non-productive cysts, and assuming the distribution to be normal; the results may be of some interest.

Analysis of the variance showed that the mean square between groups was considerably greater than the mean square within groups, the variance ratio being 6·5. This is highly significant; however, only the differences between the 100 earthworm series and the other treatments are more than twice the standard error obtained from the error mean square. Treatment of the data in two groups, an 'earthworm group' containing all the different earthworm series, and a control group, gave a standard error of ±17·25; with a difference between the two means of 53·2, this is highly significant ($p = 0{\cdot}001$).

With material of this type, giving a very much skewed distribution, a logarithmic transformation is often useful. Mr D. J. Finney, of the Rothamsted Experimental Station, very kindly carried out an analysis of variance using such a transformation. In order to avoid difficulties with the zero cysts, the quantity $\log(x+1)$, where x is the number of larvae per cyst, was used in the analysis. The results are given in Table 2; the mean values shown are, essentially, the logarithms of the geometric means. Neither analysis of variance, including or excluding zeros, shows any significant effect of treatments,

TABLE 2. *Soil experiments. Mean values of* log $(x+1)$ *and* S.E., *where* x = *total larvae emerging, per cyst*

	Earthworms per pot				
	100	60	40	20	Control
All cysts, i.e. incl. zeros	1·171 ±0·137	1·164 ±0·168	0·889 ±0·168	1·207 ±0·168	1·038 ±0·119
Productive cysts only, i.e. excl. zeros	1·882 ±0·109	1·746 ±0·128	1·668 ±0·144	1·575 ±0·120	1·518 ±0·090

although when zeros are excluded, the mean square is greater than that for error. Counting all cysts, the table of means shows little regularity; excluding non-productive or 'zero' cysts, the number of larvae increases regularly, as shown in Fig. 1, from the controls up to the 100-earthworm pot; this linear trend is highly significant ($p<0{\cdot}01$).

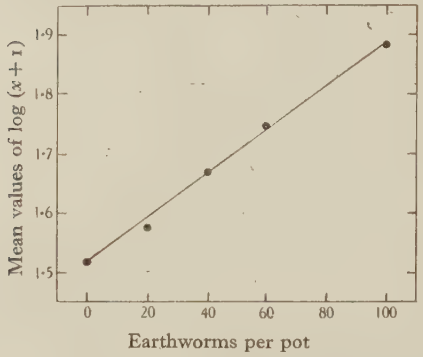

Fig. 1. Emergence of eelworm larvae and number of earthworms in soil; productive cysts only. Logarithmic transformation of numbers of larvae per cyst to quantity log $(x+1)$ where x is the number of larvae per cyst. Mean values plotted are essentially logarithms of the geometric means.

(2) *Larvae per day*

Fig. 2 shows the emergence per day for the pooled data of the 40- and 60-pots and controls; differences in total number of larvae produced have been eliminated by recording the emergence per day as a percentage of the total. The treated cysts respond more rapidly to the stimulus of potato root excretions. The 20- and 100-earthworm series were started first and, as counts of larvae were first made at 3-day intervals, no data are available for the 4th and 5th days. Nevertheless, they too showed, in a more general way, the same difference in rate of response.

These experiments indicated that the earthworms had two effects; first, they appear to cause more larvae to hatch, and, secondly, these larvae seem to emerge more rapidly. On these two points, particularly the latter, it seemed necessary to obtain more information. Accordingly, a second series of experiments was undertaken in July and August 1943.

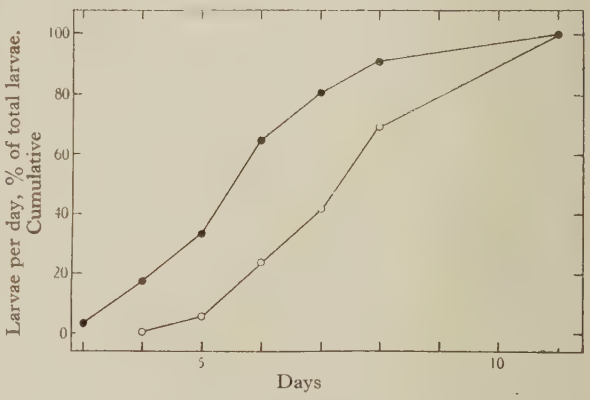

Fig. 2. 'Soil' experiments: emergence of eelworm larvae per day. ● experimental; ○ control.

B. CAST EXPERIMENTS

Materials and methods

It seemed to be particularly desirable to examine cysts which had passed through the digestive tract of earthworms. Accordingly, earthworms were kept in moist silver sand for 5 days; their casts were then free from soil. Twenty were then placed in about an inch of moist silver sand in each of two $3\frac{1}{2}$ in. plant pots, the worms being completely covered. Eelworm cysts were separated from soil by the usual flotation method, and spread on the surface of the sand together with the debris which floated with them. The pots were then filled with more silver sand. Casts were collected each day from the surface of the sand for a fortnight. They were then air-dried for 5 days, and the cysts separated from them; 71 cysts were thus obtained.

Tests were carried out with root excretions as before, the experimental cysts being compared with controls. Larvae were counted every day from the third day until emergence was slight, and then every 2 or 3 days until it had completely ceased.

Results

Larvae per day. The results of the 'cast' experiments confirm the suggestion of the June experi-

ments. Table 3 shows that the eggs in the experimental cysts respond very much more rapidly than the controls. To eliminate differences due to total

TABLE 3. *'Cast' experiments. Larvae emerging per day*

Day	3	4	5	6	7	8
Experimental	61	505	817	305	111	99
Control	3	20	57	210*	133	173
Day	9	10	12	14	15	17
Experimental	83	48	28	13	3	0
Control	188	146	78	37	14	8
Day	18	19	20	22	24	26
Experimental	14	10	1	5	0	0
Control	14	16	8	20	6	21

* 115 produced by one cyst.

that very fertile cysts may have obscured the response of the others and thus give an incorrect impression. It was necessary to determine how each group of eggs in the different cysts responded to the root excretions. Accordingly the number of larvae, per day, for each individual cyst, was expressed as a percentage of the total number of larvae it produced, e.g. with a total of 50 larvae produced, 10 of these on the 6th day would equal 20 %. This eliminates fertility from consideration; it also heavily weights the data in favour of the less fertile cysts, and therefore is strictly incorrect mathematically. Nevertheless, it will serve a useful purpose by testing whether the response is confined to the very fertile cysts; cysts producing less than 5 larvae were, however, eliminated from these somewhat arbitrary calculations as possessing undue weight, leaving 30 experimental and 31 control cysts. The totals for each day

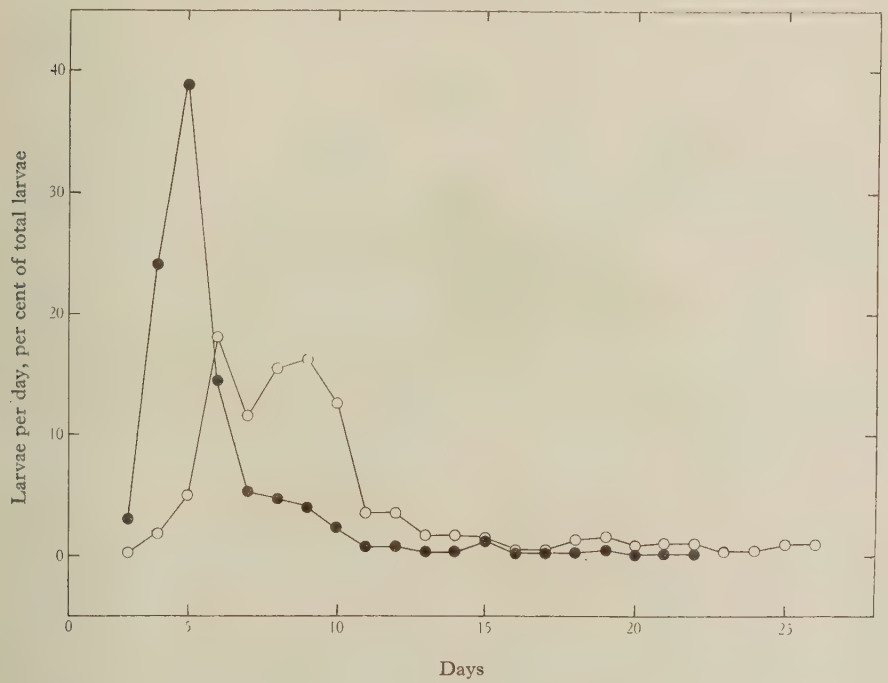

Fig. 3. 'Cast' experiments: emergence of eelworm larvae per day. ● experimental; ○ control.

number of larvae produced, the number of larvae, per day, has been expressed as a percentage of the total; these values are shown in Fig. 3. The difference between the two series is very striking; by the 5th day, 65 % of all larvae of the experimental series have emerged, but only 7 % of the controls. The response appears to be qualitatively different.

Although the results are striking, it is possible, with data based on a heterogeneous cyst population, are plotted in Fig. 4; they are corrected for the difference in cyst number, and expressed for convenience in percentile units. The experimental series shows a sharp peak on the 5th day, with very little emergence on other days; the controls show a much greater spread. The data for the 5th and 9th days, the peaks for experimental and controls respectively, are presented in Table 4. The experimental series includes cysts producing from 5 to 276 larvae;

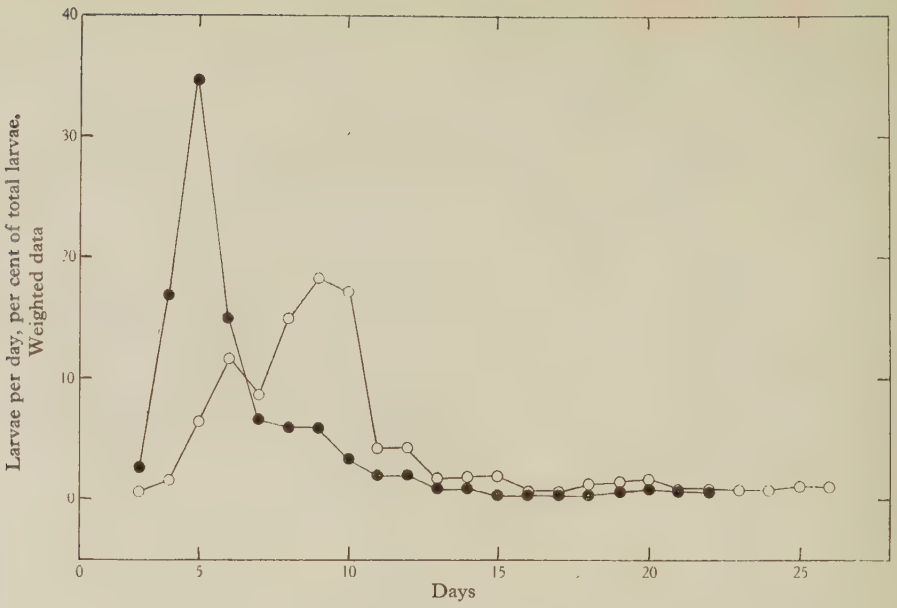

Fig. 4. 'Cast' experiments: emergence of eelworm larvae per day—'percentage' data—see text. ● experimental; ○ control.

TABLE 4. *'Cast' experiments. Emergence of larvae per day as a percentage of total emergence, per cyst. 5th and 9th days only*

Experimental			Control		
Total	% 5th day	% 9th day	Total	% 5th day	% 9th day
7	14·3	0	20	0	30·0
19	52·7	0	17	0	23·6
7	42·9	0	63	0	23·8
54	51·8	3·7	76	22·4	1·3
97	47·4	3·9	11	9·1	27·3
7	57·1	0	69	0	14·5
5	40·0	0	26	19·2	7·7
67	0	13·4	8	12·5	0
61	65·6	1·6	90	0	20·0
78	57·7	0	52	0	25·0
204	34·8	0	49	0	10·2
105	40·0	1·9	33	0	21·2
13	7·7	23·1	24	0	62·5
83	36·2	3·6	43	0	30·2
39	43·6	5·1	18	0	27·8
276	43·5	0·7	16	12·5	6·3
62	43·6	6·5	8	25·0	37·5
9	0	11·1	28	17·9	21·4
267	33·8	5·2	49	0	8·2
50	8·0	16·0	8	0	25·0
9	0	22·2	6	16·7	16·7
19	47·4	0	19	36·7	5·3
225	31·6	4·0	250	0	11·6
34	17·7	5·9	42	4·8	19·1
24	29·2	33·4	16	50·0	0
160	64·4	1·9	14	0	35·8
50	46·0	0	9	22·2	11·1
15	40·0	0	10	0	20·0
5	20·0	0			
25	32·0	8·0			

the agreement among them in hatching rate is therefore remarkable. Out of 26 cysts producing larvae on the 5th day, only five produce less than 30 % of their total; the mean value for that day is $34{\cdot}5 \pm 18{\cdot}8$. Only 12 control cysts produce larvae on the 5th day; 2 of these produce more than 30 % of their total, and the mean value is $6{\cdot}1 \pm 17{\cdot}36$. The control cysts reach their maximum about the 9th day; 26 cysts produce larvae, and the mean is $17{\cdot}5 \pm 14{\cdot}1$. The difference in the value of the S.D., relative to the mean, on the 5th and 9th days, for experimental and controls respectively, will be noted; clearly the response of the former is almost in the nature of a trigger reaction.

Larvae per cyst. Results are presented in columns *a* of Table 5; columns *b* will be referred to later. The number of larvae produced is considerably lower than in June, for both experimental and control groups, the corresponding mean values being about 50 % lower in both cases; this, presumably, is connected with the so-called dormant period of the eelworm. As in June, the most fertile cysts are found only in the experimental group, with a unique cyst among the controls producing 250 larvae. The variation is great; assuming a normal distribution, the difference between the means, including all cysts is 18·1; with a standard error of $\pm 7{\cdot}58$, this difference is significant ($p = 0{\cdot}02$). Excluding non-productive cysts, the difference between the means is 25·6; with a standard error of $\pm 12{\cdot}03$, this difference is significant, although barely so ($p = 0{\cdot}05$). With a logarithmic transformation, the differences between the means, either including or excluding

TABLE 5. *'Cast' experiments. Total number of emerging larvae (a) and full eggs remaining (b), per cyst*

Experimental								Control							
a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1	320	7	79	0	0	1	1	3	14	—	6	20	84	17	267
39	8	0	0	1	0	276	51	63	303	76	43	1	0	0	11
62	60	9	298	267	54	0	0	11	345	3	16	0	140	69	210
50	239	9	70	1	2	19	84	26	110	12	23	0	0	8	1
225	*	34	*	24	*	1	1	8	382	90	329	52	206	49	214
160	27	2	2	0	0	50	120	0	11	33	152	24	1	2	92
15	26	5	151	1	187	3	16	0	7	2	11	1	9	0	0
0	52	0	0	0	0	25	95	43	215	0	0	18	193	0	70
19	154	0	0	0	0	7	1	4	80	0	0	0	0	0	233
0	0	4	214	54	65	0	4	16	157	8	9	0	0	28	110
1	95	97	275	7	9	0	0	3	184	49	220	8	53	2	233
0	46	0	0	5	2	1	4	1	77	6	76	4	8	4	38
0	0	3	64	67	280	0	0	19	172	1	15	0	3	0	83
2	10	61	90	78	134	204	0	0	107	5	5	0	0	0	1
0	0	0	0	0	0	105	3	0	0	1	10	0	0	0	23
1	0	1	0	1	2	0	26	250	184	42	23	0	0	4	106
13	12	83	109	3	74	0	0	0	0	16	120	14	118	4	121
0	0	0	1	0	0	—	—	0	1	1	1	1	14	0	191
								0	56	9	67	4	42	0	0
								0	260	3	36	0	0	0	0
								0	190	10	35	0	57	0	0

Mean number of larvae per cyst and S.E.:

All cysts	$31{\cdot}8 \pm 5{\cdot}58$	All cysts	$13{\cdot}7 \pm 5{\cdot}13$
Productive cysts only	$48{\cdot}1 \pm 8{\cdot}7$	Productive cysts only	$22{\cdot}5 \pm 8{\cdot}3$

Log transformation. Mean value of log $(x+1)$:

All cysts	$0{\cdot}628 \pm 0{\cdot}079$	All cysts	$0{\cdot}762 \pm 0{\cdot}086$
Productive cysts only	$1{\cdot}034 \pm 0{\cdot}087$	Productive cysts only	$1{\cdot}151 \pm 0{\cdot}091$

* Cysts lost.

TABLE 6. *'Cast' experiments. Larvae, eggs, cysts; summary*

	Egg-containing (E.-C.) cysts	Productive cysts	Empty cysts	Eggs hatched (= larvae produced)	Eggs remaining	Larvae produced per E.-C. cyst, and S.E.	Log transformation. Mean values of log $(x+1)$
Experimental	52	47	19	1974	3747	$40{\cdot}6 \pm 7{\cdot}06$	$1{\cdot}04 \pm 0{\cdot}121$
Control	71	51	13	1148	6978	$16{\cdot}2 \pm 6{\cdot}05$	$0{\cdot}743 \pm 0{\cdot}103$

zeros, are little greater than the S.E. (calculations kindly carried out for me by Mr D. J. Finney). Clearly, the variation in the data is too great. Yet treatment of larvae, in terms of 'larvae per cyst' may obscure the true nature of the response; the relevant fact is the number of larvae which hatch in proportion, not to the cyst, but to the total number of larva-containing eggs originally present. It was therefore necessary to investigate the number of full eggs remaining in the experimental and control cysts.

Eggs per cyst. The cysts were dissolved in hypochlorite solution (Smedley, 1936) and the eggs still containing larvae counted; the results completely transform the data. They are summarized in Table 6, the values for each cyst being shown in columns *b* of Table 5. The largest single change is brought about by the differences in the numbers of empty cysts in the experimental and control groups, 19 out of 71 in the former and 13 out of 84 in the latter; a χ^2 test showed that this difference was not statistically significant (p = about 0·1). Excluding empty cysts from consideration, 47 out of the 52 in the experiment group, but only 52 out of the 71 in the control group produced larvae; this difference is significant ($p = 0{\cdot}02$). In larvae produced per cyst, the differences between the two series are highly significant; with natural numbers, $p = 0{\cdot}01$, with the logarithmic transformation, $p = 0{\cdot}001$. But the striking nature of the earthworm influence is most clearly shown in a comparison of the numbers of larvae hatching in relation to the number of larvae present in the eggs at the beginning of the hatching tests; 1148 out of 8124 (14 %) for the control series, and 1974 out of 5721 (34·5 %) for the experimental series. An elaborate test is hardly necessary to test

the significance of such a difference; χ^2 is almost 800, an extremely significant value. While the 19 larvae-containing cysts of the control group which failed to produce larvae contained 1451 larvae, only 129 larvae were contained in the 5 similar cysts of the experimental series, further evidence of the difference between the two groups. The solitary cyst of the control series which produced a large number of larvae contained by far the largest number of eggs, and equal numbers of larvae were produced by cysts of the experimental series which contained far fewer eggs. Using the percentage hatch of the eggs of the control cysts as a standard, it can be estimated that the number of eggs hatching from the experimental series under normal conditions would be about 800; the earthworms thus increase the hatch by about 145 %. It may not be without significance that this is of the same order of magnitude as the highest values obtained by Boyd (1943) with dilute solutions of certain organic silver compounds.

Discussion

The 'cast' experiments clearly show that earthworms have a profound influence on the eggs contained in eelworm cysts; on subsequent stimulation by potato root excretions more eggs hatch, and they hatch more rapidly; these two effects may be independent. A third point, presumably following from the first, is that more cysts produce larvae. Apparently the eggs are of three kinds: those which would have hatched in any case, those which would not have hatched unless influenced by earthworms, and those which did not hatch during the experiments. The absence of the first category from some cysts accounts for the fact that significantly more cysts of the experimental series produced larvae. Cysts of this type will, presumably, produce fewer larvae than the other productive cysts, so that differences based on the means of groups of productive cysts will tend to be reduced in magnitude. This point is of some importance in relation to the results of the 'soil' experiments. Nevertheless, it is clear that, as the 'larvae-producing' cysts of an experiment are not the only ones capable of producing larvae, the exclusion from calculations of non-productive cysts is unjustifiable.

It is interesting to compare the extent of the differences between the experimental and control series estimated with the different standards available. There are four standards: (1) number of eggs hatching in proportion to the number of eggs present, (2) number of eggs hatching, per cyst, excluding empty cysts, (3) number of eggs hatching, per cyst, including all cysts taken, i.e. including 'zero' cysts, (4) number of eggs hatching per cyst, excluding non-productive cysts, i.e. excluding 'zeros'. The estimated percentage increases in number of larvae produced as a result of earthworm action are respectively as follows: 145, 149, 117, 99 %. The first of these standards is, clearly, the correct one; the cyst basis approaches it closely if the empty cysts are excluded, but the other estimates are far from the truth. The single cyst technique possesses considerable advantages over multiple cyst methods, and the present work, for example, could only have been carried out by this method; yet it focusses even more attention on the cyst as the unit, whereas the contained eggs constitute the reacting system, and columns *a* and *b* of Table 5 show how they may vary in number and physiological state from cyst to cyst. Clearly, comparisons based on numbers of emerging larvae, per cyst, may give quite erroneous impressions. Nevertheless, it is doubtful if determination of the number of eggs per cyst, as a routine measure, could be carried out, as it is far too laborious; it should not be impossible, however, to examine the non-productive cysts only, so as to eliminate empty cysts from the calculations. Selection of cysts prior to an experiment, so as to eliminate empty cysts, is to be avoided as it may lead to unconscious selection of cysts on other grounds.

The results of the 'cast' series of experiments are so decisive that they confirm those of the earlier, 'soil', series; the progressive increase in the number of emerging larvae, per cyst, with increase in the number of earthworms per pot, may therefore be taken as genuine, a result which is of particular relevance to field aspects of the problem. Particular importance is also attached to these results because the cysts, in the different pots, will have been under identical conditions, save for the variations in number of earthworms per pot. The cysts of both 'cast' and 'soil' of experiment were obtained from the same soil sample, so that it can be assumed that the egg content of both was of the same type. Judging by the maximum egg content of the 'cast' series, the hatches of many of the cysts of the 'soil' series must have approached the 100 % level.

It is difficult, from the present series of experiments, to say with certainty that the changes in the physiological state of the eggs result from the passage of the cysts down the digestive tract of earthworms. In the 'cast' series of experiments, although the possibility of cysts being worked to the surface by the bodily activities of the earthworms cannot be completely excluded, it is certain that the vast majority of the cysts examined were derived from genuine casts. Nevertheless, if it were shown that the eggs are influenced by some substance produced in the soil or sand by the earthworms, the response of cysts derived from casts would not invalidate the hypothesis.

A number of nematode worms habitually parasitize earthworms so that the gut content of the latter must be suited to the hatching of certain nematode eggs; the response of eelworm eggs may be related to this. On the other hand, the increase in number of emerging larvae, per cyst, with increasing num-

bers of earthworms per pot, may be thought to suggest that the concentration of some substance in the soil in concerned; this theory would not necessarily conflict with the view that a digestive enzyme plays some part, for enzymes have been isolated from the body wall of earthworms (Lesser & Taschenberg, 1908). Alternatively, this progressive increase may be caused by larger numbers of cysts passing through the gut in the pots with many earthworms, or, perhaps, by passage more than once. Whatever the cause, it is clear that the effects persist for a considerable time, for cysts from the 'soil' series of experiments were exposed to the air for some months before the hatching tests. The effect on potato plants, however, is problematical, for it is by no means certain that a heavy attack of shorter duration, which, presumably, would result, would be more difficult to withstand than a more prolonged attack of lower intensity.

Moltz (1932) and Smedley (1936) have shown that cysts soaked in dilute solutions of bleaching powder produced more larvae than controls on subsequent immersion in root excretions; these solutions will also stimulate hatching alone. Hurst (1938) and Fenwick (1943) show that dilute solutions of picric acid will stimulate hatching in the absence of root excretions, while Boyd (1943) shows that, after soaking in dilute solutions of certain organic silver compounds, eelworm cysts in root excretions produce more larvae than controls. There are, then, a number of cases in which chemical substances have been shown to have a stimulating effect on eelworm eggs. But this is the first occasion, so far as I am aware, on which a naturally occurring agent has been shown to have a somewhat analogous effect. As it is by no means clear from the present work whether the influences demonstrated were produced by passage through the gut of earthworms or in some other way, it would be advisable to reserve judgment on the mechanisms involved; but clearly, if the results presented in the present paper are substantiated by further work with cysts of different origins, some of the perplexing behaviour of the potato root eelworm may become more clear.

I wish to thank Prof. A. D. Hobson for his interest in the work, and Mr D. J. Finney, for advice on the statistical treatment of some of the data.

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The behaviour of mercury compounds in soil*

By J. R. Booer, Ph.D., B.Sc.

(With 9 Text-figures)

The uses of inorganic compounds of mercury for the control of plant pests is reviewed, and a summary of the relevant chemical and physical properties of the compounds concerned is given. On chemical evidence a working hypothesis is propounded showing that all compounds may be expected to decompose into metallic mercury.

A pot technique is described by means of which a correlation can be obtained between the effective mercury content of a given soil sample and the rate of growth of wheat seedlings. The mathematical treatment of the results is described, and the validity of the pot technique is verified by statistical analysis of results. Using the pot technique it is shown that volatilization losses are insignificant but that mercury is slowly rendered ineffective by the formation of mercuric sulphide. The effect of sulphur-reducing bacteria is considered and the influence of *Vibrio desulphuricans* on mercury is studied in detail.

Experimental evidence obtained by the pot technique is produced to show that mercurous chloride slowly decomposes in the soil giving mercury and mercuric chloride, mercuric chloride rapidly decomposes into mercury and mercurous chloride, and other inorganic compounds decompose directly into mercury. The working hypothesis is substantiated in all major aspects.

The uses and properties of the organo-mercury compounds are then discussed. Type compounds selected are ethyl mercury phosphate, phenyl mercury acetate and methoxyethyl mercury acetate.

Using the pot technique it is shown that the formation of organo-mercury clays takes place and that these clays decompose giving metallic mercury. A mechanism is suggested.

Introduction

In the *Journal of Agricultural Science*, 1935, **31**, p. 632, the writer undertook to describe the chemical properties of mercury phenate, a compound which at that time showed promise as a seed disinfectant. Although mercury phenate was later superseded, the need still remained for a description of the chemical and physical properties of the organo-mercury compounds used in plant pathology, and it became apparent that very little information was available on the reactions with which the agricultural scientist is mainly concerned, namely, those which take place in the soil. A review of the literature revealed the necessity for an investigation of these soil reactions but failed to disclose any suitable technique. No progress in this matter was therefore possible until a study of the effects of metallic mercury led to the development of the pot technique which is described here. The work on metallic mercury led automatically to a study of the inorganic compounds of mercury, and in due course a connecting link with the organo-mercury compounds was found. The work is therefore presented in logical as well as in chronological order.

During the past decade there has been a marked increase in the total amount of mercury applied to the soil for the control of agricultural and horticultural pests, but as the manufacture of mercury compounds is in the hands of a limited number of specialists no data are available from any country on the amounts of mercury used in this manner. The quantities are, however, larger than might be supposed, and in various forms the use of mercury compounds in agriculture and horticulture is international. The increasing and persistent use of mercury in this way has led plant pathologists, bearing the toxicity of mercury compounds in mind, to ask the following questions:

When mercury compounds are applied to the soil, what changes do they undergo?

Are the effects of mercury compounds temporary or permanent?

Is mercury a cumulative soil poison?

If not, what factors affect its elimination or what processes render it innocuous?

One of the objects of this work is to provide the answers to these questions.

The uses of inorganic compounds of mercury as pest control agents

The fungicidal properties of mercuric chloride solution were first reported by Bolley (1891), whose recommendations formed the basis for the method, now used in many parts of Canada and the U.S.A., for the control of *Rhizoctonia* disease of potatoes (*Corticium solani*). By this method 'seed' potatoes are soaked for 90 min. in a cold aqueous solution of 1 g. mercuric chloride/l., and the amount of mercury

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transferred by the potatoes to the soil is about ½ lb./acre. A modification of this method, introduced by Cunningham (1925) and later critically examined by Plummer & Bonde (1941), consists of the use of a mercuric chloride solution, 2 g./l., acidified with hydrochloric acid, and the period of immersion is reduced to 5 min. From the data available, this method slightly reduces the amount of mercury applied to the soil.

Another modification consists of the use of an aqueous suspension of 8 g. of yellow mercuric oxide/l. This is known as the 'instantaneous dip' method and is popular only in the U.S.A. The amount of mercury transferred to the soil is probably between 1 and 2 lb./acre.

It is reasonably certain that more mercury is transferred to the soil by the disposal of spent solutions and sludges from these dipping operations than by planting the treated potatoes.

The unpublished work of Hiltner (*c.* 1912) at Munich, with which the author is acquainted and which is mentioned by Martin (1940), gave rise to the production of several inorganic mercury preparations for the treatment of cereal and vegetable seeds. Most of these have since been superseded by the organo-mercury seed disinfectants, but a proprietary preparation containing 6 % mercuric chloride and 2 % mercuric iodide as its active ingredients continues in successful use in the African cotton-growing districts. This preparation is used for the control of the bacterial disease, black arm or angular leaf spot of cotton (*Xanthomonas malvacearum*), and is a dust which is applied to the seed. The treatment results in the application of 0·01 lb. mercury/acre.

The work of Glasgow & Gloyer (1924) laid the foundation of the method of controlling club root disease of Brassica crops (*Plasmodiophora brassicae*) by means of a mercuric chloride solution. The method which is recommended in many countries consists of applying ½ pt. of an aqueous solution of 1 g. mercuric chloride/l. to the planting hole shortly before transplanting. At the average plant population for such crops, about 8000 plants per acre, this method results in the addition to the soil of about 3½ lb. mercury/acre.

The use of mercurous chloride (calomel) as an insecticide was first reported by Glasgow (1929) whose investigations led to the use of calomel in the U.S.A. for the control of the cabbage root maggot *Delia* (*Hylemyia*) *brassicae*. As practised in America, this method consists of applying a dust containing 10–50 % calomel in a suitable inert filler to the roots of the seedling when transplanting. Wright (1938) investigated this subject still further and developed the method recommended in England for the control of cabbage-root maggot of applying a dust containing 4 % calomel in an inert filler on the soil at the base of the plant. The rate of application is about 3 g. of dust per plant, and sometimes a second application is made within 2–3 weeks. The English method entails the addition of 2–3 lb. mercury/acre with a plant population of 8000. For the control of cabbage-root maggot in seed beds, Wright (1940) recommended the use of 1 lb. of 4 % calomel dust/60 row yards. At 12 in. spacing between rows this corresponds to the application of about 8 lb. mercury/acre.

Dustan (1936) studied the use of calomel for the control of onion fly, *Delia* (*Hylemyia*) *antiqua*, and this subject was also further investigated by Wright (1939) with the result that two methods were adopted in England. One method consists of treating onion seed with calomel, with the aid of an adhesive paste. The rate of application is 1 lb. calomel per lb. of seed, and as the rate of seeding may be of the order of 7–30 lb./acre, the method entails the application of 6–25 lb. mercury/acre. The alternative method for the control of onion fly employs the 4 % calomel dust previously mentioned, applied along the rows at seedling emergence. The amount of dust applied is about 350 lb./acre, and as two applications are generally made, the method results in 25 lb. mercury/acre being added to the soil.

Preston (1928) first reported the control with calomel of club root disease of cabbages, and his subsequent investigations (Preston, 1942) led to the method employing 0·5 g. calomel diluted with inert filler applied to the planting hole when transplanting. This operation involves the application of about 8 lb. mercury/acre.

In the U.S.A. and Canada, various mercury compounds have been used for the control of the turf diseases, brown patch and dollar spot caused by *Rhizoctonia* and *Fusarium* spp. Monteith & Dahl (1932) reported on many such treatments, the most popular of which employs an intimate mixture of two parts of mercurous chloride and one part of mercuric chloride. This mixture, either suspended in water or diluted with sand or soil, is applied at the rate of 2 or 3 oz./1000 sq. ft. As many as three treatments may be applied in one year, entailing the addition of perhaps 5–20 lb. mercury/acre.

An operation of some economic importance in parts of the U.S.A. is the control of common scab of potatoes, *Actinomycetes scabies*, by incorporating 5 lb. of yellow mercuric oxide per ton (2000 lb.) of compound artificial manure. Such mixtures are not usually broadcast but are applied in drills between the rows of potatoes at rates up to 1000 lb./acre.

An obsolete method for the eradication of earthworms from turf and nursery beds consists of applying liberal quantities of an aqueous solution containing 0·5–1 g. mercuric chloride/l. Non-poisonous preparations of Mowrah meal have displaced this method.

It will be observed that, with one exception,

although the amount of mercury applied per acre is relatively small, all methods involve application to restricted areas. Thus, Wright's method for controlling cabbage-root maggot entails the application of 0·1 g. mercury over an area of about 12 sq. in., which would be 112 lb. mercury/acre if the whole area were treated. In this case the calomel is applied to the surface of the soil which remains undisturbed for an appreciable time. This example serves to show that in most cases a high concentration of mercury is produced locally, and this important fact forms the basis of the pot experiments described later.

The exception is the method of controlling turf diseases. Here the maximum uniformity of distribution is required, but the same area, such as a golf green, may be treated for several years in succession. It is therefore probable that over a period of 3 years, 45 lb. mercury/acre will be applied. There is thus no fundamental difference in this apparent exception.

The removal of mercury from the soil

Three methods are available for the removal of mercury from the soil, namely, leaching, removal by the crop, and volatilization.

No information is recorded on the effect of rainfall on mercury compounds in the soil. The only observation bearing on the subject is by Stock & Cucuel (1934) who regard mercury as an omnipresent element, and give the mercury content of natural waters as 0·01–0·05 μg./l.

Winkler (1935) has described a method for the estimation of mercury in leafy vegetables, but little is recorded on the mercury content of treated plants. Stock & Cucuel (1934) also state that plants may contain up to 4 μg./100 g. On this basis the average cabbage crop would remove no more than 0·4 g. mercury/acre. On general grounds there is little reason to believe that the crop removes much mercury from the soil. Mercury compounds precipitate many proteins and are therefore incompatible with living plants. It is, moreover, well known that excessive doses of mercury compounds can cause clearly defined deformities. These deformities do not occur when plants are treated by the prescribed methods. On the contrary, the treated plants, doubtless as the result of pest control, are generally more robust and healthy.

Zimmerman & Crocker (1934) made extensive investigations showing that the addition of mercury compounds to the soil results in the liberation of mercury vapour in quantities sufficient to produce toxic concentrations in confined spaces such as greenhouses. These workers were, however, more concerned with aerial effects and did not investigate the soil reactions or the proportion of the added mercury compounds converted into the metallic state and liberated as such.

It will thus be seen that the available information on the elimination of mercury is very incomplete.

The chemical and physical properties of inorganic compounds of mercury

This review of chemical and physical properties is limited to that range of conditions which appertains to the cultivation of crops. Thus, extremes of temperature and pressure beyond the limits occurring in nature can be ignored and no mention need be made of reactions which, for obvious reasons, are not likely to occur in the soil.

Mercury. The outstanding physical property of the metal is that it is volatile at all relevant temperatures, even at temperatures below its melting point, minus 39·7° C. *International Critical Tables* (1928) give the vapour pressure of mercury as 0·000185 mm. Hg at 0° C. and 0·006079 mm. Hg at 40° C. The rate of evaporation of mercury is known to be governed mainly by the surface exposed but is also influenced by the purity of the metal. It has been recorded that mercury will volatilize through a layer of water, although the weight of evidence tends to show that the element is completely insoluble in water.

Mercury is a relatively stable metal and is easily produced by the gentle reduction of many of its compounds. There are however a number of slow reactions which might be expected to take place under soil conditions.

Although water, oxygen and carbon dioxide separately have no effect on mercury, moist air slowly converts it into mercurous oxide. At 40° C. sulphur combines readily with mercury, but the reaction has not been investigated at lower temperatures. In the presence of oxygen, hydrogen sulphide converts mercury into mercuric sulphide. This reaction is retarded, but not suspended, by the presence of water. Dilute acids and alkalies are without action on mercury.

Mention should be made of the very great affinity of mercury for sulphur. All inorganic compounds of mercury yield mercuric sulphide in the presence of hydrogen sulphide or alkali sulphides.

Mercury behaves both as a monovalent and as a divalent metal, forming respectively mercurous and mercuric compounds.

Mercurous chloride (*calomel*), HgCl. Much of the published information on the physical properties of mercurous chloride should be treated with reserve, as many investigators have failed to recognize the fact that the method of preparation may profoundly influence the physical properties.

The older method of precipitation from solutions of mercurous nitrate and sodium chloride has been largely replaced by the direct chlorination method. The various conditions under which this method can be carried out give rise to a wide range of particle

sizes and porosities, and investigators would be well advised always to ascertain the exact nature of the material they use.

Mercurous chloride can be described as insoluble in water, unaffected by air or oxygen, and quite stable in the dry state. The effect of water on mercurous chloride has, however, been the subject of much controversy, but it can definitely be stated that the modern type of mercurous chloride dissociates into mercury and mercuric chloride. Little is known on the speed of this reaction but experience in the removal of mercuric chloride by washing with distilled water for the production of pharmaceutical calomel has shown that the dissociation is accelerated by increased temperature, by agitation and by the presence of alkali halides. Hobson (1941) has shown by inference that the dissociation of mercurous chloride is accelerated by wool. Dilute acids are without action on mercurous chloride, but alkalies cause superficial blackening with the formation of mercury and mercurous oxide. Calcium carbonate is without action unless heated in the presence of water. Hydrogen sulphide and alkali sulphides cause superficial blackening with the formation of mercuric sulphide.

Mercuric chloride (*corrosive sublimate*), $HgCl_2$. The solubility of mercuric chloride in water is 3·5 % at 0° C. and 5·4 % at 20° C.: it can therefore be regarded as readily soluble.

It is unaffected by air or oxygen, and mild alkalies precipitate mercuric oxychloride. A small addition of hydrogen sulphide to an aqueous solution of mercuric chloride produces a white precipitate, and further additions ultimately result in the formation of black mercuric sulphide. Alkali sulphides behave similarly.

Mercuric chloride is a strong oxidising agent. Among the substances recorded as reducing it are alcohol, sugar, gelatine, many organic acids, and the decoctions of various plants, as recorded by Nevins (1838). The chief product of the reduction is always mercurous chloride, but some mercury may also be formed. It is also known that in Kyan's timber preservation process by which timber is impregnated with a dilute solution of mercuric chloride, much reduction to mercurous chloride takes place, and the mercury retained in the timber is mainly in the form of mercurous chloride.

Mercuric oxide, HgO. Of the two forms of mercuric oxide only the yellow variety need be considered. It is doubtful whether mercuric oxide can exist in the soil for any appreciable time. In the first place it forms mercuric chloride and caustic soda in contact with a dilute solution of sodium chloride. Secondly, it acts as a powerful oxidising agent on many organic compounds, being reduced to mercury or mercurous oxide in the process.

Mercurous oxide, Hg_2O. This compound need not be considered in any detail. It is very unstable and behaves as a mixture of mercury and mercuric oxide.

Mercuric oxychlorides. These compounds also do not require detailed consideration since, within the limits of this investigation, they can be regarded as mixtures of mercuric oxide and mercuric chloride.

Other soluble mercury compounds. Although other soluble mercury compounds are not normally used for the control of pests, a reference to them is included to complete the picture from the chemical standpoint.

In the mercurous series, the only soluble compound likely to be encountered is the nitrate. It is to be expected that soluble chlorides in the soil will immediately precipitate mercurous chloride.

Of the soluble mercuric compounds, the most important are the nitrate and the acetate. Both these compounds are much more soluble in water than mercuric chloride, and it is to be expected in both cases that with soluble chlorides in the soil they will yield mercuric chloride.

Mercuric phosphate, $Hg_3(PO_4)_2$. In view of the possibility of this compound being formed in the soil, a reference to its properties is included.

In cold dilute solutions of mercuric chloride and di-sodium phosphate acidified with nitric acid, mercuric phosphate is precipitated only very slowly. The salt is usually prepared from mercuric nitrate solution from which it precipitates more readily. It is insoluble in water.

There is insufficient chemical evidence to establish whether mercuric phosphate would be formed in the soil. It has already been shown that mercuric chloride may be reduced at an uncertain rate, and this reaction may be more rapid than the precipitation of mercuric phosphate. Further investigation of this point is justified in view of the importance of phosphates in plant nutrition.

Mercury sulphides. The existence of a mercurous sulphide is still open to doubt, and substances purporting to be mercurous sulphide are unstable at temperatures above 0° C.

The white precipitate sometimes obtained in the early stages of passing hydrogen sulphide through mercuric chloride solution is often described as mercuric oxytrisulphide $HgO.3HgS$. It is a controversial point whether this substance is a compound, but if so, the linkage is feeble and it can be regarded here as a mixture.

Black mercuric sulphide, HgS, is the most stable of all the mercury compounds. It is almost completely insoluble in water, and is unaffected by water, air and dilute acids or alkalies. It is, in fact, one of the most stable of the metallic sulphides. At soil temperatures mild oxidising or reducing agents have no action. It is soluble in solutions of the alkali sulphides, but a high concentration of the latter is necessary to have any marked effect. Thus a litre of

solution containing 7·8 g. sodium sulphide dissolves 1·91 g. mercuric sulphide, and such conditions are not likely to be encountered in the soil.

The working hypothesis

Using the available chemical data, it is possible to formulate a working hypothesis, which is given in diagrammatic form in Fig. 1.

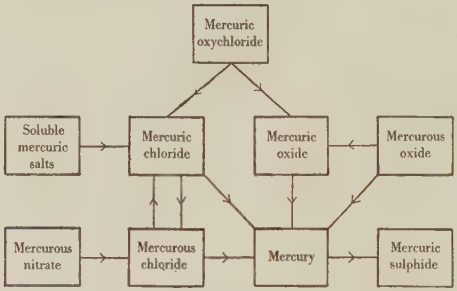

Fig. 1.

The essential points in the hypothesis are as follows:

(1) The dissociation of mercurous chloride into mercuric chloride and mercury.

(2) The reduction of mercuric chloride to mercurous chloride and mercury.

(3) The conversion of mercury into mercuric sulphide.

As chemical methods are unsuitable or not available for the investigation of the hypothesis, it has been necessary to use biological methods.

The pot technique

In 1939, preliminary field trials were made on the control of club root disease in cabbage seed beds with a so-called zinc amalgam dust containing 2 % zinc dust and 6 % finely divided mercury mixed with a siliceous filler. This is a preparation developed by Daines (1936) who has expressed the opinion that the active fungicide in soil treatments is metallic mercury. When this dust was applied to the seed drill at the rate of 1 lb./40 yd., the emergence and early growth of the seedlings were seriously retarded. As the treatment gave excellent disease control, investigations were begun with the object of eliminating the retardation, the first step being to ascertain its cause. The effect was therefore reproduced with cabbage seed in standard seed boxes, and it was found that the presence or absence of the zinc made no difference to the retardation. To reduce the size and increase the convenience of the experiments, seed boxes were replaced by glass crystallizing dishes, and it was observed that finely divided metallic mercury retarded the early growth of cabbage seedlings without reducing the percentage germination. Finally, cabbage was replaced by wheat which appeared to react similarly and had the great advantage of being convenient for daily measurement.

At this stage, no reference could be found in the literature to this phenomenon, although Woodman *et al.* (1934) had recorded that treatment of soil with mercuric chloride solution retarded the early growth of Brussels sprouts seedlings, and this they attributed to the formation of mercury clay.

It has been found that the rate of growth of wheat seedlings can be correlated with the mercury content of a given sample of soil, and the following technique has been used.

The tests were carried out in glass crystallizing dishes 12 cm. diam. and 7 cm. deep. These were filled with soil to a depth of 5 cm., corresponding to about 500 g. soil, and in each experiment uniformity of replicates was obtained by weighing the soil. In the case of mercury compounds which are fine powders, the treatment of the soil was effected by first mixing the compound with nine times its weight of fine silica. This mixture was diluted with about 20 g. of the soil and then thoroughly mixed with the remainder of the soil. Water-soluble compounds require a modification of the technique which is described under the appropriate headings.

Before use, the soil was air-dried and sifted through a sieve of 10 meshes per linear inch. In addition to purified sterile sand, two types of soil have been mainly used in this investigation. Soil A was a light sandy loam from the Croydon, Surrey, district, and was taken from a vegetable garden which had been in normal cultivation for about ten years. It was deficient in humus and lime, giving a reaction of *p*H 6·0. Soil B was obtained from one of the main dykes on Romney Marsh, Kent. This soil was rich in humus and clay, and deficient in lime, giving a reaction of *p*H 6·5.

The soil having been prepared, 150 c.c. was removed before sowing the seed. The soil remaining in the dish was levelled and pressed down gently, and a measured volume of water was added through a sprinkler. Wheat of high germination was used, and 25 seeds were arranged in a uniform 5-square pattern, the distance between seeds being about 1 cm. The remainder of the soil was then replaced gently, levelled and pressed down, giving a planting depth of 1·4 cm., and a further measured quantity of water was added. The amount of water added, being a significant factor, is discussed in greater detail later.

The majority of the pot tests were carried out at room temperatures between 55 and 65° F. Under these conditions the seedlings emerged in 4–7 days, and thereafter daily records of emergence were made and the length of each seedling measured to

the nearest millimetre. The mean length of the seedlings was recorded.

Of the various possible methods of presenting the results the one selected as being the most concise and accurate was to determine by arithmetical interpolation the mean height of the treated seedlings at the point of time when the mean height of the appropriate control was 6 cm. The validity of this procedure is apparent from the results of a typical experiment with soil treated to contain 0·01 % Hg, shown graphically in Fig. 2: both are sigmoid curves

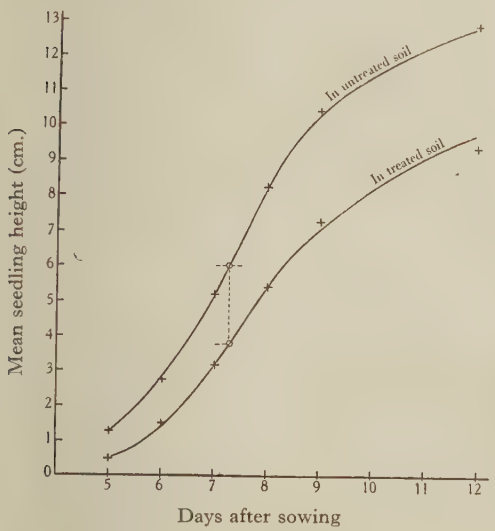

Fig. 2. Effect of 0·01 % Hg in soil.

whose points of inflexion approximately synchronize. In the curve for the untreated soil the point of inflexion lies very near the 6·0 cm. value and growth is practically linear between the two adjacent recorded values.

Several important advantages accrue from the selection of the mean seedling height of 6 cm. as the standard. By the time the seedlings have reached this height, the variations in the mean caused by uneven emergence have disappeared. The seedlings can be easily counted and measured, and the measurement is not complicated by the second leaf which is usually evident in some seedlings by the time the mean has reached 8 cm. It has been found in repeated sets of measurements on the same pot that the mean can be determined with an accuracy of ±0·01 cm.

The following experiment is described in detail, as it serves to illustrate the method and to establish the degree of accuracy which is obtainable.

The effect of an addition of 0·01 % metallic mercury to 500 g. samples of soil A was studied: 140 c.c. of water was added and six replicates of each treatment were made.

The results were as follows:

	No mercury		0·01 % mercury	
Replicate no.	5th day cm.	6th day cm.	5th day cm.	6th day cm.
1	3·2	8·1	1·7	5·8
2	2·9	7·7	1·5	5·1
3	2·9	8·3	1·6	5·9
4	2·8	7·9	1·3	5·3
5	2·9	8·3	1·7	5·8
6	3·0	7·9	1·6	5·9

By arbitrarily selecting Replicate No. 1 as the control for the entire experiment, and replacing the graphical method used in Fig. 2 by a simple calculation, it can be shown that a mean seedling height of 6·0 cm. was reached in 5·57 days, the process being as follows:

	cm.
Mean height on 6th day	8·1
Mean height on 5th day	3·2
Growth in 1 day	4·9

Growth required from 5th day = 6·0 − 3·2 cm. = 2·8 cm.
Time required for growth of 2·8 cm. = 2·8/4·9 = 0·57 days
∴ Total time required to reach 6·0 cm. = 5·57 days

By similar calculations, the results at 5·57 days can be re-stated as follows:

Replicate no.	No mercury cm.	0·01 % mercury cm.
1	6·0	4·0
2	5·7	3·6
3	6·0	4·1
4	5·7	3·7
5	6·0	4·1
6	5·8	4·1

If these results are regarded as means and tested for significance as such by the usual method, the statistics obtained are $n = 10$, $t = 17·8$, $P < 0·01$.

If, as was the case in this experiment, the soil used for each pair of replicates is intimately mixed before adding the mercury to the treated portion, and the seed intimately mixed before sowing, the reduction in height can be used as a basis for examining the results. The figures are then as follows:

Differences

Replicate no.	cm.
1	2·0
2	2·1
3	1·9
4	2·0
5	1·9
6	1·7

Statistical analysis of these figures gives the following results: $n = 5$, $t = 32·6$, $P < 0·01$, and on

this basis, the least significant difference at $P=0{\cdot}01$ is 0·22 cm.

Thus, the pot technique gives very consistent results, and a concentration of 0·01 % mercury can be detected with ease. The detection of lower concentrations appears to be well within the sensitivity of the test.

Glass vessels were chosen in order to eliminate any possible variation in the evaporation of mercury. The apparatus also has the advantage that evaporation can, if desired, be entirely eliminated by covering the dish with a tightly fitting lid.

It should be emphasized that the growth of wheat seedlings is used solely as a quantitative reaction, using the word in the sense familiar to the chemist. The explanation of this phenomenon is a matter for the plant physiologist, for whose information the following notes are recorded.

Solutions of mercury compounds coming into direct contact with seeds can cause severe damage to germination. Contact with metallic mercury or mercury vapour, however, causes no damage to the percentage germination of wheat, and no abnormal growth can be observed. Only the rate of growth is reduced and the fresh weights of the seedlings both above and below ground are less when mercury is present in the soil. Wheat germinated without soil or sand in glass vessels in the presence of mercury germinates at the same rate as when no mercury is present, and if seeds germinated in the presence of mercury are transferred to normal soil they grow at the normal rate. Wheat germinated in glass vessels in the absence of mercury and transferred to a soil containing mercury grows at a subnormal rate.

It has been repeatedly observed that in soil containing mercury the rate of emergence is lower than in the control. This is explained by the lower rate of growth, as the seedling has to attain a height of 1·4 cm. above the point of planting before it is visible above ground.

Since the above observations were made, Gray & Fuller (1942) have recorded that, although mercury in the soil delays the germination of various vegetable seeds, the final germination percentage is unaffected.

The behaviour of metallic mercury in soil

This aspect of the subject is considered first in view of the chemical evidence tending to show that many mercury compounds may decompose into the metal when brought into contact with soil.

The effect of various amounts of mercury in soil A is shown graphically in Fig. 3, in which the ordinates are the mean seedling heights in centimetres as determined by the pot technique, and the abscissae are the logarithms of the percentages of mercury in the soil.

Additions of potash, nitrates and phosphates in substantial doses were found to be entirely without influence on the effective mercury content. These observations establish that the action of mercury is specific.

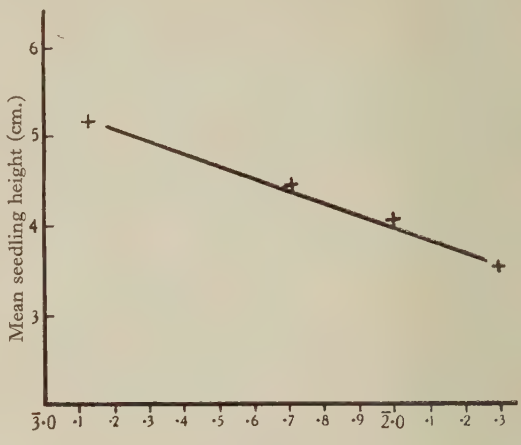

Fig. 3. Effect of metallic mercury

The pot technique was used to study the volatilization of mercury from the soil. Zimmerman & Crocker (1934) have recorded that mercury vapour can be detected by the gold leaf method above soils treated with mercury compounds. A simpler method is to expose selenium sulphide paper, which is stained black by minute quantities of mercury vapour. The atmosphere above soil to which 0·01 % mercury had been added darkened selenium sulphate paper rapidly, but 3 months' storage of this treated soil in glass dishes exposed to free ventilation produced no significant reduction in the effective mercury content provided the amount of water in the soil was insufficient to cause waterlogging. The conclusion reached at this stage is that volatilization is not a major factor in the elimination of mercury from the soil.

By the time the investigations had reached this point, it was recognized that the application of mercury to the soil might produce a form of soil poisoning which could extend beyond the period of pathological benefit. Methods of eliminating the poisoning were therefore studied and, of many treatments investigated, only two were found to give positive results. These were:

(*a*) The addition of powdered sulphur to the treated soil. Under moist, aerobic conditions the addition of 0·2 % sulphur to soil A, whilst producing no immediate effect, completely eliminated in 4–7 days the retardation of growth resulting from the addition of 0·02 % mercury.

(*b*) Waterlogging the soil to produce anaerobic conditions resulted in a gradual elimination of the retardation effect.

Probably the most striking feature of soil under anaerobic conditions is the readiness with which hydrogen sulphide is formed. It is also formed under aerobic conditions in the presence of elemental sulphur. Therefore, the most tenable theory, explaining both phenomena, is that hydrogen sulphide converts metallic mercury into mercury sulphide. In further confirmation of this theory, it has been established by pot tests that as much as 0·2 % mercuric sulphide in the soil is without effect on the rate of growth of wheat seedlings.

Mercury and the sulphur cycle

The formation of hydrogen sulphide in the soil under either aerobic or anaerobic conditions involves biological factors, and it is therefore necessary to consider the position of mercury with respect to the sulphur cycle of the soil. The latter has been fully described by Bunker (1936), from whose publication Fig. 4 is reproduced by permission of H.M. Stationery Office.

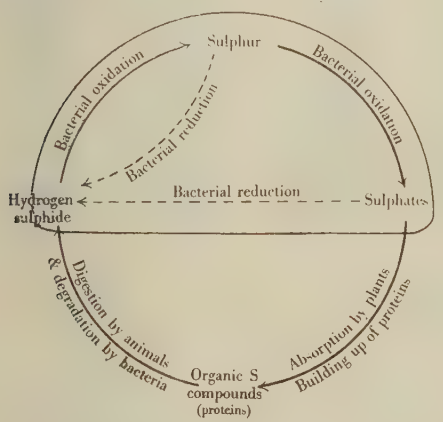

Fig. 4.

There are three phases of the cycle in which the interaction of mercury and hydrogen sulphide might take place, these being:

(1) The bacterial reduction of sulphur.
(2) The bacterial reduction of sulphates.
(3) The degradation of proteins.

Of these, the effect of mercury on the degradation of proteins in the soil has not yet been investigated. The influence of the bacterial reduction of sulphur on mercury is mentioned here for the first time, and has not yet been fully studied. The relation between mercury and the bacterial reduction of sulphates has however received some attention and a description of the work is now given.

The anaerobic reduction of sulphates is brought about by the organism *Vibrio* (*Sporovibrio*) *desulphuricans*. This can easily be cultured by means of the sulphate reduction medium described by Galloway & Burgess (1937), and having the following composition:

Distilled water	1000 c.c.
Di-potassium hydrogen phosphate	0·5 g.
Ammonium chloride	1·0 g.
Calcium sulphate	1·0 g.
Magnesium sulphate	2·0 g.
Sodium lactate	3·5 g.
Ferrous ammonium sulphate	0·5 g.

If samples of soil are put in test tubes, the soil covered with sulphate reduction medium, and the tubes closed with rubber bungs, the development of the organism can be readily detected by the blackening of the soil, following which black colonies appear in the supernatant medium. Additional refinement is obtained by inserting a plug soaked in alkaline pyrogallol before closing the tube, and whilst the optimum temperature is 30° C., the organism multiplies rapidly at room temperatures.

Although *Vibrio desulphuricans* is omnipresent, Bunker (1939) has recorded that the population density varies considerably. Using the simple technique described, it was observed that soil B was much richer in *V. desulphuricans* than soil A, and the former was therefore used in the following experiments. Preliminary tests showed that the addition of either mercury, mercurous chloride or mercuric chloride retarded the growth of *V. desulphuricans*, but did not kill it. A quantitative test was therefore made, as follows.

10 g. samples of soil B, previously air-dried and sifted, were placed in ½ in. test tubes. 6 c.c. of mercuric chloride solution was added to each tube, this being just sufficient to saturate the soil. 10 c.c. of sulphate reduction medium was then added and the tubes were closed. The strengths of the mercuric chloride solutions ranged from 1:1000 to 1:6000 with a control.

At the beginning of the experiment the soil was light brown, but after 6 days at room temperature, blackening was observed starting at the bottom and progressing slowly upwards. By the seventh day the soil in the control tube was completely blackened. The condition of the tubes after 23 and 35 days respectively is shown in Figs. 5 and 6. It will be seen that *V. desulphuricans* can survive in the presence of mercury, and in this connexion it should be noted that mercuric chloride solution is used as a soil fungicide at strengths between 1:1000 and 1:2000.

An experiment was then carried out to ascertain whether the toxic effect of a given quantity of metallic mercury could be affected by conditions favouring the growth of *V. desulphuricans*. 0·1 g. mercury was mixed with 10 g. of heat-sterilized and unsterilized soil B respectively. An untreated, unsterilized control was included. Sulphate reduction

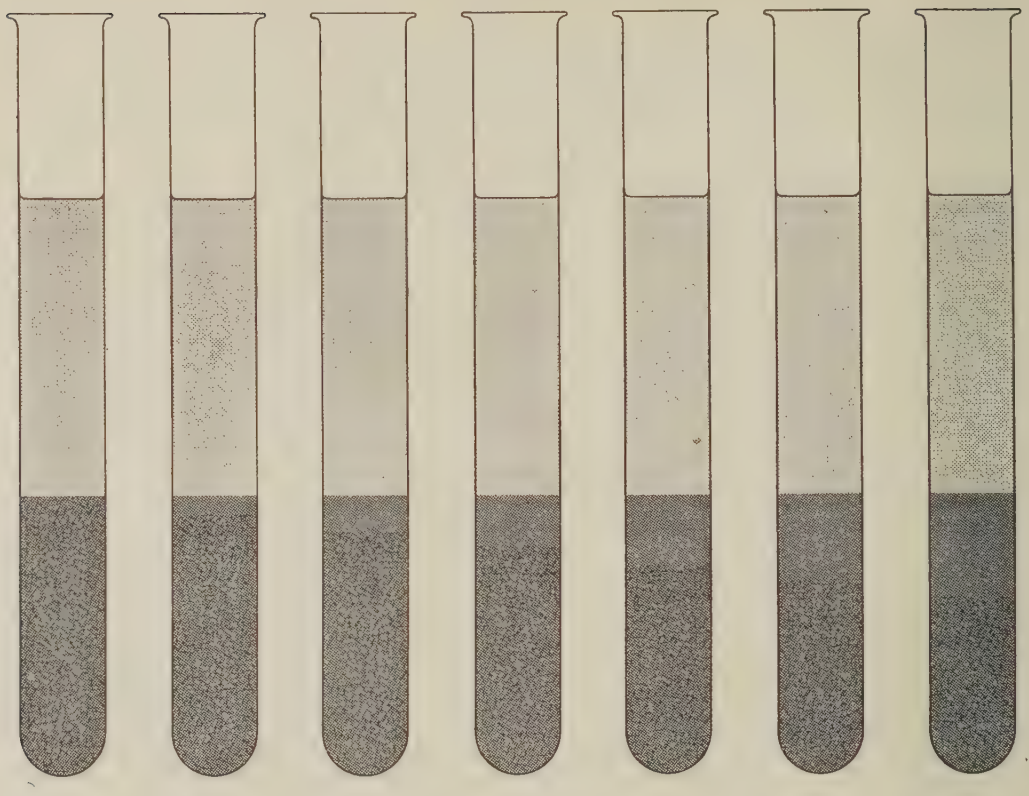

Fig. 5.

medium was added and in 28 days the control and the unsterilized soil containing mercury were completely blackened. Very slight darkening was observed in the sterilized soil, but the sterilization of *V. desulphuricans* by heat is notoriously difficult and it was not found possible to obtain complete sterility without using temperatures which might have affected the chemical composition of the soil. The contents of the tubes were then transferred to 500 g. samples of soil A with which they were intimately mixed, giving a concentration of 0·02 % mercury in the treated soil. The pot technique was then applied, with the following results:

	cm.
Control	6·0
Mercury in 'sterile' soil B	3·6
Mercury in unsterilized soil B	5·3

Reference to Fig. 3 shows that contact with *V. desulphuricans* has reduced an initial concentration of 0·02 % mercury to less than 0·001 %, a removal of at least 95 % of the effective mercury, under conditions where all variables except the formation of hydrogen sulphide have been eliminated.

Attempts to reach finality by producing a sample of pure mercuric sulphide by the action of *V. desulphuricans* on mercury have, unfortunately, not yet been successful. In the absence of such convincing proof, it is not possible to go beyond the statement that there is strong evidence supporting the view that the elimination of mercurial poisoning of the soil in conditions favouring the production of hydrogen sulphide is due to the formation of mercuric sulphide.

The behaviour of mercurous chloride in soil

The working hypothesis is based on the assumption that the reaction

$$Hg_2Cl_2 \rightarrow HgCl_2 + Hg$$

takes place slowly in the soil, and mention has already been made of the fact that the only influence likely to accelerate the reaction in the soil is an increase in

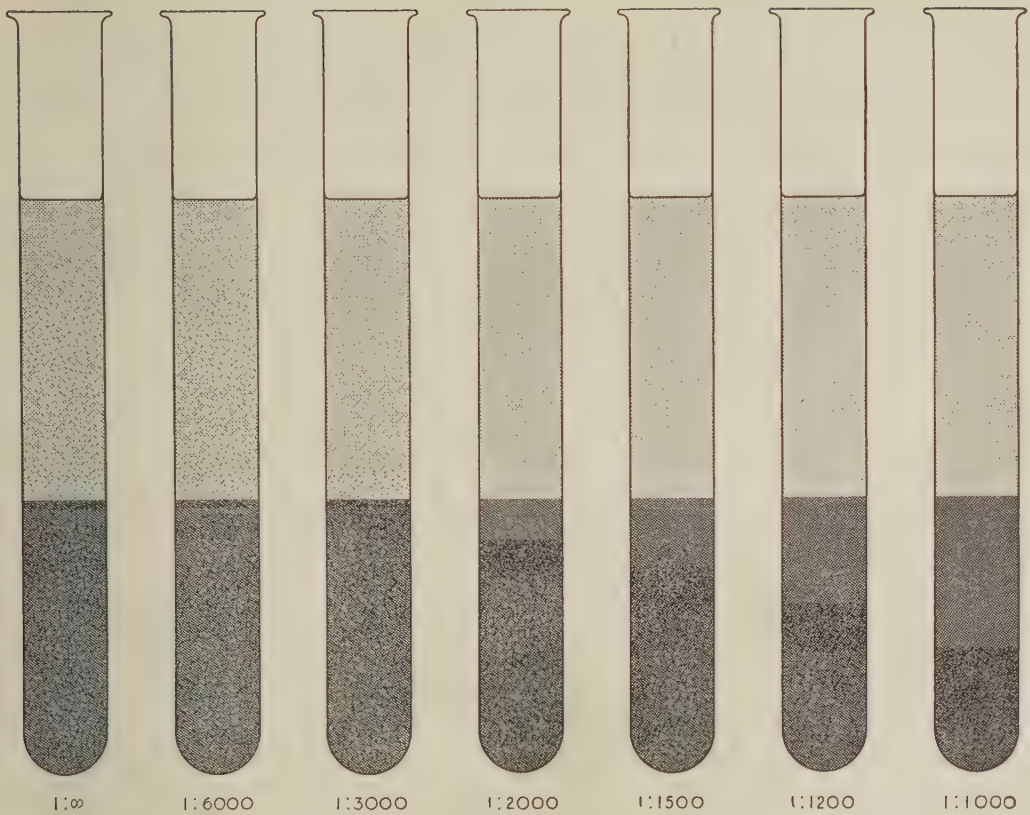

Fig. 6.

the electrolyte concentration. If the working hypothesis is correct, the following effects should be observed.

(1) The effective concentration of mercury as determined by the pot technique should increase progressively.

(2) The effective concentration of mercury should be increased by the addition of an electrolyte such as sodium chloride.

In the foregoing it is assumed that any mercuric chloride formed is very rapidly converted into mercurous chloride and/or mercury. Evidence in support of this assumption is presented in the section dealing with mercuric chloride.

The effect of various concentrations of mercurous chloride in soil A is recorded in Fig. 7, which also shows the effect of storing the calomel-treated soil for various periods before sowing the seed. These experiments show that the anticipated increase in the effective mercury concentration does take place over a relatively long period.

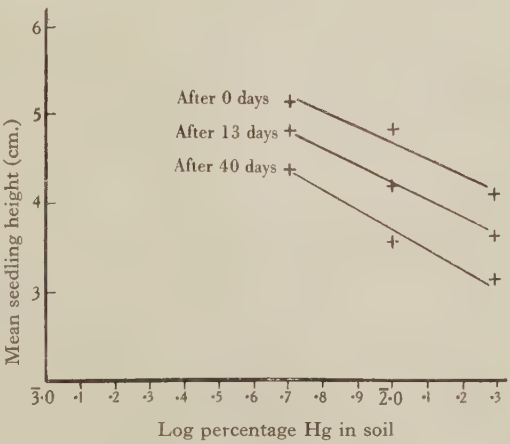

Fig. 7. Effect of mercurous chloride.

The effect of increasing the sodium chloride content of the soil is demonstrated in the following experiments which are designed to eliminate both the specific action of sodium chloride and any effect it might have on metallic mercury. Replicated samples of 500 g. each were prepared with soil A, as follows:

No. 1. Soil A (Control).

No. 2. Soil A plus 0·1 % sodium chloride.

No. 3. Soil A plus 0·005 % mercury.

No. 4. Soil A plus 0·005 % mercury and 0·1 % sodium chloride.

No. 5. Soil A plus 0·012 % mercurous chloride.

No. 6. Soil A plus 0·012 % mercurous chloride and 0·1 % sodium chloride.

The seed was sown immediately after preparing the soil samples to which 140 c.c. of water was added, and the following results were obtained by the pot technique. Sample no. 1 was used as the control for samples nos. 3 and 5 to which no salt was added. To eliminate the action of salt sample no. 2 was used as the control for samples nos. 4 and 6.

		Without salt cm.	With salt cm.
Mercury free	No. 1	6·00	
	No. 2		6·00
Mercury	No. 3	3·75	
	No. 4		3·50
Mercurous chloride	No. 5	4·20	
	No. 6		3·20

A similar experiment using 0·04 % sodium chloride gave similar results and it may thus be concluded that whilst sodium chloride has no significant effect on the action of metallic mercury, it causes a marked increase in the effective concentration of mercury produced by the decomposition of mercurous chloride.

The validity of this portion of the working hypothesis is thus established.

It may be asked whether the pot technique is recording the effect of mercury or of mercurous chloride, and on this point there appears to be no doubt. There is no reason why the effect should increase over a period of time if it were produced by mercurous chloride, and although pure mercurous chloride produces no staining of selenium sulphide paper, mercury vapour is readily detected in this way when moist soil is mixed with calomel. It may be added that wheat seedlings from the calomel-treated soil resemble those from mercury-treated soil in that there is a complete absence of any obvious deformation.

The fact that mercurous chloride can in certain circumstances produce a greater effect than that produced by an equivalent amount of metallic mercury, requires an explanation. It is not claimed that the pot technique can be used to determine an absolute concentration of mercury. It is reliable only for comparing differing amounts of mercury in the same form. The effects recorded in Fig. 3 were produced by a mechanical mixture of mercury and fine silica, and the individual mercury particles were doubtless relatively coarse. The mercury produced by the decomposition of calomel is first formed as vapour and then condensed, and may therefore be expected to be in a much finer state of subdivision than in a mechanical mixture. It has already been recorded here that the rate of evaporation of mercury is influenced by the surface exposed and therefore this apparent anomaly is actually further evidence in support of the working hypothesis.

The behaviour of mercuric chloride in soil

It is well known that solutions of mercuric chloride can exert a powerful phytotoxic action, and the work of Munro & Newton (1934) may be quoted as an example of this knowledge.

It was observed that if the pot technique was carried out with pure sand, no seedlings grew when a 0·005 *M* solution of mercuric chloride was applied. If, however, soil was used instead of sand the seedlings grew, albeit somewhat abnormally, even if no precautions were taken to prevent the solution coming into contact with the seed. This observation not only serves to justify the modification of the pot technique which is described later in this section, but clearly indicates that a very rapid reaction takes place between the soil and the mercuric chloride solution. This reaction has already been recognized empirically, and its recognition is apparent in the frequent recommendation to apply mercuric chloride solutions to the soil shortly before planting or sowing. For instance, in Northern Ireland it is recommended for the control of club root disease that seed beds should be watered with mercuric chloride solution 1:1500 'just before' the seed is sown. Proof of the rapidity of this reaction was obtained during the investigations on *V. desulphuricans*. A 1:2000 solution completely kills this organism in the absence of soil, whereas Figs. 5 and 6 show that in the presence of soil B this strength produces no more than a slight retardation. Attempts have been made to measure the speed of this reaction, but the inevitable time lag in the pot technique renders the method unsuitable. The tests that have been made, however, show that the reaction is complete in less than one hour, and for the purpose of the present work it may therefore be regarded as instantaneous.

In determining the effect of various amounts of mercuric chloride in soil, the following modification of the pot technique was used. 100 g. of air-dried, sifted soil A was treated with 10 c.c. of mercuric chloride solution, and sufficient time was allowed for the solution to be completely absorbed. This

treated soil was then mixed with a further 400 g. of air-dried, sifted soil A, after which the pot technique was carried out in the manner already described. The strength of the mercuric chloride was adjusted to give the required dosage, and the results obtained are shown graphically in Fig. 8.

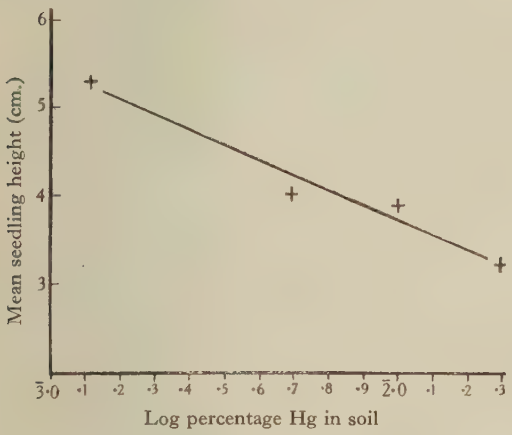

Fig. 8. Effect of mercuric chloride.

The working hypothesis admits the possibility of both mercurous chloride and mercury being formed by the decomposition of mercuric chloride, and no chemical method exists for distinguishing between these two substances in the presence of soil. The investigations on the behaviour of mercurous chloride have, however, shown that the presence of this compound in soil can be detected by the addition of sodium chloride. The following experiment was therefore carried out. Replicated 500-g. samples of soil A were treated with mercuric chloride solution to give a concentration of 0·01 % mercury in the soil. After being allowed to dry, the treated soil was mixed with 0·2 g. sodium chloride. Controls comprised untreated soil, soil treated with salt, and soil treated with mercuric chloride only. After eliminating the salt effect as before, the results were as follows:

	Without salt cm.	With salt cm.
Control	6·0	6·0
Mercuric chloride	4·3	3·7

It has already been demonstrated (see Fig. 7) that the effective mercury content produced by the addition of mercurous chloride increases as the treated soil is stored. This evidence has been used to confirm the fact that the mercuric chloride is decomposed into mercurous chloride in an experiment in which soil freshly treated with 0·01 % Hg as mercuric chloride was compared with a similar sample that had been stored in a moist condition for 29 days, with the following results:

	cm.
Control	6·0
Freshly prepared sample	3·6
Sample stored 29 days	3·1

It is clear from both experiments that mercurous chloride is present, and whilst it is not possible to arrive at a precise estimate, it would appear from a comparison of these results with those quoted under the heading of mercurous chloride that under the conditions of these experiments and with soil A, the major proportion of the mercuric chloride is converted into mercurous chloride on coming into contact with the soil.

The behaviour of mercury oxides in soil

The chemical evidence on which the working hypothesis is based indicates that both mercuric oxide and mercurous oxide will decompose rapidly in contact with the soil, yielding mercury. If this is correct the pot technique should show no difference between mercury, mercuric oxide and mercurous oxide at equal mercury concentrations. In the following experiment a concentration of 0·01 % mercury was produced by the addition of equivalent amounts of the three substances:

Replicate no.	Control cm.	Mercury cm.	Mercuric oxide cm.	Mercurous oxide cm.
1	6·0	3·75	3·70	3·90
2	6·0	3·55	3·60	3·50
3	6·0	3·85	3·75	3·95
Mean	6·0	3·72	3·68	3·78

The close agreement of these results substantiates this portion of the working hypothesis.

The behaviour of mercuric oxychloride in soil

Mercury oxychloride is included in this investigation, not because it is used in plant pathology, but because it might be formed when mercuric chloride solution is applied to soils which are alkaline either as the result of recent liming or from any other cause. To substantiate the working hypothesis, it will suffice to show that the effective concentration of mercury produced by mercury oxychloride is indistinguishable from that produced by chemically equivalent weights of mercuric oxide and mercuric chloride added separately.

The mercury oxychloride used in the following tests was the stable brown form having the composition $HgCl_2.3HgO$, and produced by the partial precipitation of a solution of mercuric chloride with caustic soda solution. The precipitate was washed and dried, and diluted with finely divided silica to produce a dust containing 10 % mercury, in which

form the material was added to soil A in the usual way to give a concentration of 0·01 % mercury.

Similar samples of soil were prepared by the methods already described in the appropriate sections, and the equivalent of 0·0025 % mercury as mercuric chloride, and 0·0075 % mercury as mercuric oxide were added as separate operations. The results were as follows:

Replicate no.	Control cm.	Mercury oxychloride cm.	Mercuric chloride + oxide cm.
1	6·0	4·20	3·95
2	6·0	3·70	4·15
Mean	6·0	3·97	4·05

It will thus be seen that there is no significant difference between mercuric oxychloride and an equivalent mixture of its constituents.

The behaviour of water-soluble mercury salts in soil

(*a*) *Mercuric salts*

Mercuric acetate has been selected to represent other water-soluble mercuric salts as it has occasionally been recommended for the treatment of potato tubers. It differs from mercuric chloride in the important respect that its aqueous solutions are ionized whereas those of mercuric chloride are not.

Various writers have recorded that the mercuric acetate is less phytotoxic than mercuric chloride and this observation appears to be confirmed by the following pot tests in which a dosage of 0·01 % mercury was applied.

Replicate no.	Control cm.	Mercuric acetate cm.	Mercuric chloride cm.
1	6·0	5·15	4·35
2	6·0	5·30	4·50
Mean	6·0	5·22	4·42

It was however observed that when soil treated with mercuric acetate was stored, the effective mercury concentration increased. Samples of treated soil stored for 24 days were found to have an effective mercury concentration indistinguishable from that produced by the equivalent amount of mercuric chloride under the same conditions. This proves that mercuric acetate decomposes into mercury much more slowly than mercuric chloride.

The results are thus far in accordance with the working hypothesis which suggests that other soluble mercuric salts are first converted into mercuric chloride. For this conversion to be complete, however, the equivalent amount of soluble chlorides must be available in the soil. This does not appear to be the case in soil A, and the conversion of mercuric acetate is therefore dependent upon the rate at which the limited amount of chlorides becomes available. This question of availability will be more clearly seen from reactions associated with the decomposition of mercuric and mercurous chlorides, thus:

$$HgCl_2 \rightarrow HgCl + Cl'$$
$$2HgCl \rightarrow HgCl_2 + Hg$$

Final proof of this portion of the working hypothesis can be obtained by showing that by increasing the chloride content of the soil the effective concentration of mercury produced by mercuric acetate is increased. The following experiment was therefore carried out.

	Pot test cm.
1. Control soil A	6·00
2. Soil A containing 0·01 % Hg added as mercuric chloride	3·90
3. Soil A containing 0·01 % Hg added to mercuric acetate	4·60
4. Soil A treated with hydrochloric acid 0·5 equivalent of the 0·01 % Hg added as mercuric acetate	4·10
5. Soil A treated with hydrochloric acid 1·0 equivalent of the 0·01 % Hg added as mercuric acetate	3·75

When the soluble chlorides in the soil are at least equivalent to the mercury, the conversion of mercuric acetate into mercuric chloride is complete and instantaneous. When the amount of chlorides is less than equivalent, the conversion of mercuric acetate into mercuric chloride is incomplete in the first instance but is directly related to the amount of chlorides present.

(*b*) *Mercurous salts*

Mercurous nitrate is the only important water-soluble mercurous salt, and its use in the pot technique introduces factors which have not previously arisen. There is the possibility that the rate of growth may be reduced by the removal of available phosphates as mercurous phosphate, and that stimulation may result from the increase in the quantity of nitrates in the soil. Gerhardt (1849) has recorded, and the writer has confirmed, that the addition of di-sodium hydrogen phosphate solution to mercurous nitrate solution results in the formation of a yellowish white precipitate of mercurous phosphate.

It was found that the addition to the soil of phosphate, as di-sodium hydrogen phosphate, in amounts equivalent to the mercury added as mercurous nitrate, had no influence on the result obtained by the pot technique, from which it is concluded that mercurous phosphate is not formed in the soil. The nitrate radicle can be ignored in view of the preliminary work in which it was established that the

addition of nitrates in substantial quantities did not affect the result of the pot technique.

Using mercurous nitrate to produce a concentration of 0·01 % mercury in the soil, the pot technique gave a result of 3·45 cm. as the mean of four replicates. This proves that the working hypothesis is incorrect in this case, since a result of about 4·8 cm. would be obtained if mercurous nitrate were converted into mercurous chloride.

As the work on mercuric acetate has shown that soil A does not contain soluble chlorides equivalent to 0·01 % mercury, pot tests were carried out with increased quantities of chlorides, but without significant influence on the effect produced by mercurous nitrate. The conclusion is therefore reached that mercurous nitrate is immediately decomposed into metallic mercury without the intermediate formation of mercurous chloride.

In the pot tests on mercurous nitrate 5 c.c. of decinormal solution was first added to 100 g. of soil. After mixing, this was added to a further 400 g. of soil. The initial mixing, which resembles any method likely to be used in practical horticulture, evidently brings the mercurous nitrate into contact with sufficient humus and other oxidisable matter to cause complete reduction to metallic mercury. On chemical grounds, mercurous nitrate should be a more powerful oxidising agent than mercuric chloride, which is almost instantaneously reduced in the soil. The chlorides in 100 g. of soil A are equivalent to less than one-fifth of the mercury added, and it is clear that if mercurous chloride is formed at all, the amount would be too small to detect by the pot technique in the presence of a concentration of metallic mercury approaching 0·01 %.

Discussion of the work on inorganic compounds of mercury

The experimental evidence obtained by the pot technique substantiates the working hypothesis with regard to all the relevant compounds except mercurous nitrate. It has thus been established that all the mercury compounds investigated, other than mercuric sulphide, decompose in the soil, yielding metallic mercury as the penultimate decomposition product. The facts are now presented in diagrammatic form in Fig. 9, which differs from Fig. 1 only in respect of the position of mercurous nitrate.

It has also been shown that in conditions which would give rise to the formation of hydrogen sulphide in the soil, metallic mercury is converted into mercuric sulphide, which is innocuous. This effect has also been observed in mercury-treated soils which have been waterlogged. In certain soils, as much as half the effective mercury content can be eliminated if the soil is waterlogged for a week, but this effect requires further study as its intensity varies considerably in different soil samples. The work which has been done, however, points to the conclusion that the formation of mercuric sulphide is the means by which the toxic effect of mercury is eliminated from soil under field conditions.

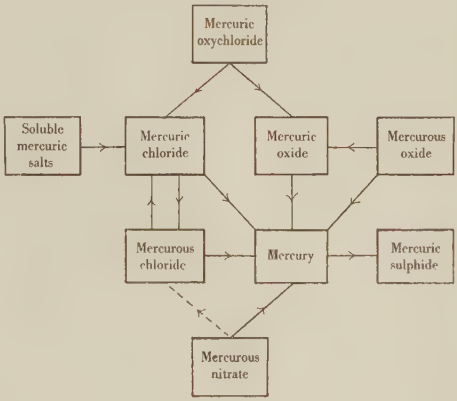

Fig. 9.

A reference should be made to the mercury clays which some workers believe to exist. The evidence is all against the existence of such substances, but it must be remembered that the clay complex of natural soils is never pure. It would appear that the reduction of solutions of inorganic compounds of mercury, possibly by humus, takes place in preference to the formation of mercury clays by base exchange. This view is supported by the low degree of ionization of such mercury compounds and their relative instability in the presence of reducing agents.

The uses of organo mercury compounds in plant pathology

The remarkable fungicidal properties of certain organo mercury compounds were first recorded by Gassner & Esdorn (1923) whose work on the *dosis curativa* and *dosis tolerata* is still regarded as a classic. The modern organo mercury dry seed dressing had already appeared in 1921 and from that time onward only a few compounds were used, in consequence of the demand for a low *dosis curativa* associated with a high *dosis tolerata*. At present all the compounds in use lie within two sections of one group, in which the relation between fungicidal power and chemical composition has been investigated by Dillon Weston and Booer (1935).

The use of organo mercury compounds in plant pathology is at present restricted to the control of numerous seed-borne fungus diseases, and although wet and dry treatments are in use, the latter are of far greater economic importance. More mercury is added to the soil as dry seed dressing than in any other way.

The principal use for wet seed dressings in this country is in the treatment of 'seed' potatoes, bulbs and flax, although they are sometimes used for cereal seeds. The tuber, bulb, corm or seed is soaked in or sprinkled with a solution under specified conditions. This method is in general responsible for the application of less mercury per acre than is the dry method.

By the dry method, and under European conditions, a dust containing organo mercury compounds equivalent to 1·0–1·5 % mercury is intimately mixed with seed at the rate of 2–2½ oz./bushel of seed, which is approximately equal to 2 g. of dust/kg. of seed. Under American conditions a dust containing the equivalent of 3·0–5·0 % mercury is applied at the rate of ½ oz./bushel of seed, thus giving a similar concentration of mercury on the seed. In view of the large consumption of these dry seed dressings in all parts of the world, and the high toxicity of the mercury compounds concerned, the question of mercury being a cumulative soil poison is most frequently raised in connexion with this practice. A closer examination of the quantitative aspect of the problem is therefore necessary.

Assuming that cereal seed is sown at an average rate of 4 bushels/acre, between 2–3 g. mercury is applied per acre. Assuming also that by cultivation the mercury is ultimately distributed uniformly in the top 6 in. of soil, say 800 c.yd. weighing 1000 kg./c.yd., and that there is no elimination of mercury whatever, then one hundred years' continuous cereal cultivation using treated seed will result in a concentration of about 0·00003 % mercury in the soil. It will be found instructive to compare this figure with the concentrations mentioned in the sections dealing with the inorganic compounds.

The chemical properties of the relevant organo-mercury compounds

The compounds concerned are all members of the group represented by the general formula R—Hg—X. The majority of seed disinfectants contain compounds in which R is an otherwise unsubstituted hydrocarbon residue such as tolyl, phenyl or ethyl. The other important class of compounds is that in which R is the alkoxyethyl group R'—O—C_2H_4—, the group in which R' is methyl being chiefly in use. In both classes X is an acidic radicle, all compounds being in effect the salts derived from the hydroxide R—Hg—OH. A full description of the chemical and physical properties of these compounds is given by Whitmore (1921), and the description given here is limited to the requirements of this work.

The chemical properties of the ethyl mercury and methoxyethyl mercury radicles very closely resemble those of the sodium ion. Their hydroxides are strongly alkaline and can be neutralized by acids to form stable salts. Strong acids form salts which are not readily hydrolysed, and which are quite strongly ionized. The solubility in water of these salts also bears a resemblance to the corresponding sodium salts, the only outstanding exception being ethyl mercury iodide which is practically insoluble in water. All these compounds are appreciably volatile at ordinary temperatures.

The phenyl mercury and tolyl mercury radicles have a remarkable resemblance to silver. Their chlorides are almost insoluble and they can be determined by titration with standard thiocyanate solution. Their hydroxides can be produced, although not precipitated, by magnesium hydroxide and other stronger alkalies. Although generally much less soluble than the corresponding ethyl mercury compounds their solutions are also ionized and solubilities between 5 and 10 g./l. at normal temperatures are not uncommon.

Another remarkable feature of the ethyl, phenyl and tolyl mercury compounds is the stability of the R—Hg-radicle. Most of the pure compounds can be melted and sublimed without decomposition. For the determination of their mercury contents, destructive oxidation must be used. Boiling with concentrated sulphuric acid must be supplemented by the addition of an oxidising agent to effect complete decomposition into mercuric sulphate. Solutions of these compounds do not give mercuric sulphide when treated with hydrogen sulphide, but the white, insoluble sulphides $(R\text{—Hg})_2{=}S$.

The alkoxyethyl mercury compounds are not so stable. They are charred at temperatures above 50° C. and, although unaffected by alkalies, are quantitatively decomposed by strong acids into ethylene and the appropriate alcohol and mercuric salt, for example:

$$CH_3\text{—O—}C_2H_4\text{—HgCl} + HCl = C_2H_4 + CH_3OH + HgCl_2.$$

The only supplementary information bearing on the subject is the report of Zimmerman & Crocker (1934) that soil treated with ethyl mercury arsenate emanates vapours which produce foliage damage resembling that caused by mercury vapour. These writers however admit the possibility that the damage may be caused by the vapour of the compound itself.

Although there is inadequate evidence to formulate a hypothesis, there are several points on which additional evidence is required. These are:

(1) As ionized solutions are involved, are organo mercury clays formed by base exahange?

(2) If so, are these clays stable, and what factors, if any, affect their stability?

(3) How do the alkoxyethyl compounds differ from the hydrocarbon derivatives?

The compounds selected to represent those in general use are ethyl mercury phosphate, phenyl mercury acetate and methoxyethyl mercury acetate.

It is appropriate to record that these compounds and other members of the series are very dangerous poisons. Inhalation of the vapour causes an ultimate breakdown of the central nervous system, and research workers who are interested should acquaint themselves fully of facts before undertaking any work with them. Recent work on this subject is described by Hunter *et al.* (1940).

The behaviour of ethyl mercury phosphate in soil

The ethyl mercury phosphate used was a well-known American seed disinfectant stated to contain 5 % ethyl mercury phosphate, equivalent to 3·8 % mercury, and found by analysis to contain 3·7 % mercury, corresponding to 5·0 % $(C_2H_3.Hg)_2.HPO_4$. The preparation possesses the feline odour characteristic of ethyl mercury compounds, and in all respects conforms to the published description. It is a fine powder and was applied as such to the soil.

A preliminary experiment, using the pot technique, was carried out with soil A and a range of mercury concentrations, and gave the following results:

Hg %	Seedling height cm.	Effect on % germination	Colour of seedling
Nil	6·00	Nil	Normal
0·0015	5·25	Nil	Normal
0·0030	4·10	Nil	Normal
0·0052	3·00	Nil	Dark green
0·0075	1·65	−10	Very dark green
0·0100	1·60	−20	Very dark green

Where damage occurred, the symptoms agreed exactly with those described by Brett & Dillon Weston (1941), and this observation, together with the seedling heights, make it evident that here the pot technique is not recording the effective mercury concentration. In the case of inorganic compounds of mercury it appears reasonable to infer that the depression of the rate of growth is caused by the toxic vapour of mercury. As the ethyl mercury compounds are generally more volatile than metallic mercury, they might be expected to produce a still greater effect, but subsequent evidence does not support this deduction.

The solubility and stability of the ethyl mercury compounds suggest that leaching might be an important factor in their removal from the soil. To investigate this point, the following experiment was made.

100 g. samples of soil A were treated with various amounts of ethyl mercury phosphate, mixed to a paste with water, and transferred to a Buchner funnel and washed with 500 c.c. of water under conditions corresponding to 3 in. continuous rainfall. The cake was dried under suction, mixed with 400 g. of dry soil A, and the pot technique applied. In the results given hereunder the 'initial mercury content' is the percentage of mercury added as ethyl mercury phosphate calculated on the total 500 g. of soil. The percentages of mercury are therefore strictly comparable with those in the previous experiment.

Initial Hg concentration %	Seedling height cm.	Remarks
Nil	6·00	Normal
0·0030	3·85	Normal
0·0052	2·90	Dark green
0·0100	1·45	Very dark green

These results show that leaching has had no effect whatever, and justify the conclusion that the formation of 'ethyl mercury' clay has taken place by base exchange.

During these investigations it was observed that there was a marked tendency for the phytotoxic action of ethyl mercury phosphate in soil to decrease with time, and this was at first attributed to volatilization. This was later proved incorrect as the effect decreased progressively when moist, treated soil was stored in closed containers. The following experiment was therefore carried out.

500 g. samples of soil A were treated with ethyl mercury phosphate to give a concentration of 0·01 % mercury, placed in closed containers and moistened with 120 c.c. of water. After 14 days storage at about 60° F. the pot technique was applied and gave a mean result of 4·50 cm. This result has to be compared with figures of 1·45 to 1·6 cm. obtained from freshly prepared soil samples.

Reference to Fig. 3 shows that a mean result of 4·42 cm. was obtained at a concentration of 0·005 % mercury, which suggests that after ethyl mercury phosphate has been added to the soil half the mercury is lost and the remaining half is converted into the organic form and thence to metallic mercury.

Further evidence on this point was obtained by studying the effects produced by sulphur. It has already been shown that the addition to the soil of 0·2 % sulphur eliminates the phytotoxic effect of mercury and inorganic mercury compounds. Parallel experiments showed that sulphur had no effect on the phytotoxic action of ethyl mercury phosphate. However, when soil which had been treated with ethyl mercury phosphate, moistened and stored for 14 days, was treated with 0·2 % sulphur with a small amount of fresh soil as innoculum, the phytotoxic effect was completely eliminated.

There is thus strong evidence supporting the view that in the soil ethyl mercury phosphate is decomposed relatively quickly, half the mercury being lost and the remainder converted into the metallic state.

A possible explanation of these observations is to be found in the general reaction:

$$2R\text{—Hg—}X \rightarrow R_2Hg + HgX_2.$$

Although many examples of this general reaction are known, the literature on the subject is unsatisfactory and incomplete. It may be that, following the formation of ethyl mercury clay by base exchange, the reactions which take place are as follows:

(1) Decomposition of ethyl mercury clay into mercury diethyl and mercury clay, thus:

$$\left.\begin{matrix}C_2H_5—Hg\\C_2H_5—Hg\end{matrix}\right\rangle \boxed{\text{Clay}} \xrightarrow{*} (C_2H_5)_2.Hg + Hg = \boxed{\text{Clay}}$$

Mercury diethyl is sufficiently volatile to be lost in a short time and would thus account for the disappearance of half the mercury.

(2) Decomposition of the mercury clay by base exchange with any available electrolyte, e.g. lime:

$$Hg = \boxed{\text{Clay}} + CaO \rightarrow Ca = \boxed{\text{Clay}} + HgO$$

The decomposition of mercuric salts into mercury has already been described.

Until the properties of ethyl mercury clay have been more thoroughly investigated, the foregoing explanations should be regarded as purely tentative.

The behaviour of phenyl mercury acetate in the soil

The phenyl mercury acetate used was a pure sample (m.p. 150° C.) prepared by recrystallization from a mixture of benzene and toluene. This was diluted with finely divided silica to give a mixture containing the equivalent of 5·0 % mercury, and this mixture was applied to the soil in the following experiments.

Again as a preliminary experiment the pot technique was carried out with a range of mercury concentrations and the following results were obtained:

Hg %	Seedling height cm.	Effect on % germination	Colour of seedlings
Nil	6·00	Nil	Normal
0·003	4·25	Nil	Normal
0·005	3·80	Nil	Normal
0·010	2·00	Nil	Normal

These results confirm the generally accepted view that phenyl mercury compounds are less phytotoxic than ethyl mercury compounds and it will be noted that whilst there is a similarity in the seedling heights produced by equivalent concentrations of phenyl mercury acetate and ethyl mercury phosphate, the former does not cause damage to germination and abnormal colouring of the seedling within the range of concentrations tested.

An experiment on the effect of leaching was made on lines identical with those used in the investigation on ethyl mercury phosphate. Using phenyl mercury acetate, the following results were obtained after leaching:

Initial Hg concentration %	Seedling height cm.	Colour of seedlings
Nil	6·00	Normal
0·003	4·95	Normal
0·005	4·05	Normal
0·010	2·65	Normal

In this instance the possible formation of phenyl mercury chloride must be considered. The solubility of this compound is of the order of 1 part in 10,000 parts of cold water, and thus 500 c.c. of water would dissolve 0·05 g. phenyl mercury chloride, which is equivalent to 0·033 g. mercury or 0·0066 % mercury in the soil. The results show that leaching has not eliminated the phytotoxic effect of 0·005 % mercury, but before the formation of 'phenyl mercury' clay can be regarded as proven, the general reduction of phytotoxic action resulting from leaching requires an explanation.

The explanation is that the samples in question were allowed to dry naturally for 4 days before the pot technique was applied, and that phenyl mercury compounds decompose rapidly in the soil. The rate of decomposition was studied in an experiment in which 0·01 % mercury as phenyl mercury acetate was added to moist soil A and allowed to stand for varying periods before the pot technique was applied. The results of this experiment were as follows:

Time of storing days	Seedling height cm.
0	1·85
2	2·90
5	3·50
8	4·05
12	4·50

The results of the leaching experiment can therefore be accepted as supporting the view that phenyl mercury clay is formed, and it will be noted that the results of the storing experiment again show that the decomposition of this complex clay results in the elimination of half the mercury.

The hypothesis regarding the decomposition of ethyl mercury clay is less tenable in the case of phenyl mercury clay as mercury di-phenyl is not sufficiently volatile to be lost in so short a time. The hypothesis would however be equally applicable to both cases if mercury di-ethyl and mercury di-phenyl were not phytotoxic, and this point has not yet been investigated. Attention is again directed to the danger of such an investigation.

* The clay micelle is shown as being divalent solely for convenience of presentation.

THE BEHAVIOUR OF METHOXYETHYL MERCURY ACETATE IN SOIL

The material used was a pure sample purified by recrystallization in water. This was diluted with finely divided silica to give a mixture containing the equivalent of 5 % mercury, and this mixture was applied to the soil. The usual pot technique with a range of mercury concentrations gave the following results:

Mercury %	Seedling height cm.
Nil	6·0
0·003	5·0
0·005	4·6
0·010	3·7

All the seedlings were normal in colour and appearance and the percentage germination was unaffected.

These results closely resemble those produced by adding to the soil similar concentrations of metallic mercury. It might be assumed that methoxyethyl mercury acetate decomposes immediately into metallic mercury, but this was proved incorrect by an experiment in which 0·2 % sulphur was added to moist soil containing 0·01 % mercury as methoxyethyl mercury acetate. After 7 days' storage the phytotoxic action was not affected. It was therefore decided to use a concentration of 0·02 % mercury in subsequent experiments. This concentration gave a result of between 1·65 and 2·25 cm. by the pot technique, and thus clearly distinguishes the effect of methoxyethyl mercury acetate from that of metallic mercury, which, at a concentration of 0·02 % gives results between 3·0 and 3·5 cm.

With a concentration of 0·02 % mercury, a leaching experiment was carried out on the same lines as before. No significant effect on the phytotoxic action resulted, again indicating that the formation of an organo mercury clay takes place.

A study of the effect of storing moist soil containing methoxyethyl mercury acetate was made under conditions identical with those already described for phenyl mercury acetate. At an initial concentration of 0·02 % mercury the following results were obtained:

Time of storing days	Seedling height cm.
0	2·30
5	3·25
10	3·05
12	3·35
15	3·10

These results indicate that methoxyethyl mercury acetate is completely decomposed into the equivalent amount of metallic mercury within 5 days. This is substantiated by the fact that if soil which has been so stored is treated with sulphur, as already described, the phytotoxic effect is completely eliminated.

An apparent discrepancy in this portion of the work will be noted. Soil freshly treated with methoxyethyl mercury acetate and sulphur shows no elimination of the phytotoxic effect in 7 days, whereas soil treated with methoxyethyl mercury acetate and stored for 5 days shows a significant decrease in the phytotoxic action.

The bacteriostatic action of the mercury compound may be an important factor, but it would also appear that sulphur inhibits the breakdown of the mercury compound. No further evidence is yet available on this point.

Attention should be directed to an important difference between the methoxyethyl mercury group and the ethyl mercury or phenyl mercury group. It has been shown that the decomposition of the last two groups is accompanied by the loss of half the mercury, whereas no mercury appears to be lost when the former decomposes. The properties of the methoxyethyl mercury clay have not been sufficiently studied for any theory to be advanced on this point, but the following mechanism of decomposition appears to be possible:

(1) The formation of methoxyethyl mercury clay by base exchange with hydrogen clay:

$$2CH_3.O.C_2H_4.Hg.O.Ac + \begin{matrix} H \\ H \end{matrix}\!\!>\boxed{\text{Clay}} \rightarrow (CH_3.O.C_2H_4.Hg)_2\!=\!\boxed{\text{Clay}} + 2AcOH.$$

(2) The breakdown of methoxyethyl mercury clay to mercury clay in the presence of acetic acid, accompanied by the formation of mercuric acetate:

$$(CH_3.O.C_2H_4.Hg)_2\!=\!\boxed{\text{Clay}} + 2AcOH \rightarrow Hg\!=\!\boxed{\text{Clay}} + 2CH_3.OH + 2C_2H_4 + Hg(O.Ac)_2.$$

(3) The breakdown of mercury clay and mercury acetate on the lines already described respectively.

This matter is, however, one which clearly requires further investigation.

DISCUSSION OF THE WORK ON ORGANO-MERCURY COMPOUNDS

The outstanding feature of the work is the discovery that the organo mercury compounds decompose in soil with a rapidity that is quite unexpected. Under normal laboratory conditions these compounds can be stored for years without their purity being affected, and reference has already been made to the vigorous methods which have to be adopted

to destroy them for analysis. Yet in the soil their existence appears to be quite transient.

Reference is again made to the concentration of 0·00003 % mercury which might result from 100 years' continuous cereal cultivation with treated seed. The results show that there is no fear of permanent injury to the soil. It has been demonstrated that treatment of the soil with sulphur eliminates the effect of relatively very heavy applications of organo mercury compounds. It is reasonable to assume that the formation of mercuric sulphide would take place in the field sufficiently quickly to eliminate the phytotoxic action from season to season.

The existence of organo mercury clays, as newly recorded compounds, is established.

Conclusion

Plant pathologists have now been provided with answers to the questions set out at the beginning of this work, as follows:

(1) *When mercury compounds are applied to the soil, what changes do they undergo?*

All the mercury compounds used in plant pathology ultimately decompose into metallic mercury. Where the chloride ion is involved, the governing factor is the rate of decomposition of mercurous chloride. With other inorganic compounds of mercury direct reduction to the metal takes place. With the organo mercury compounds the rate of decomposition of the organo mercury clay is probably the governing factor.

(2) *Are the effects of mercury temporary or permanent?*

The effects of mercury are not permanent, but may last for a considerable time. The factors influencing the rate of formation of mercuric sulphide have not yet been investigated, but moisture content, temperature conditions and soil population may all be expected to play their part.

(3) *Is mercury a cumulative soil poison?*

No.

(4) *If not, what factors affect its elimination, or what processes render it innocuous?*

Mercury is not eliminated but is rendered innocuous by the formation of mercuric sulphide. This is attributed to the bacteria which give rise to the formation of hydrogen sulphide. The addition of sulphur to the soil appears to be the best method of purifying the soil after treatment with mercury.

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Proceedings of the Association of Applied Biologists

Ordinary Meeting of the Association held on Friday, 23 June 1944, at 11 a.m. at the Imperial College of Science and Technology, London; the President, Prof. W. Brown, in the Chair.

Symposium on cereal diseases

The following papers were read and discussed:

I. Cereal diseases in England and Wales. By W. C. Moore.
II. Biological specialization in the cereal rusts. By Prof. F. T. Brooks.
III. Looking back and looking forward: a retrospect of cereal diseases in East Anglia in the past 21 years. By W. A. R. Dillon Weston.
IV. Cereal diseases in Scotland. By R. W. G. Dennis.
V. Cereal diseases in Mid-Wales. By D. Walters Davies.
VI. The take-all disease of cereals. By S. D. Garrett.
VII. Eyespot, *Cercosporella herpotrichoides*, and lodging of wheat. By M. D. Glynne.

Cereal diseases in England and Wales

By W. C. Moore, *Plant Pathology Laboratory, Milton Road, Harpenden, Herts.*

Over fifty different diseases of cereals have been listed as occurring in England and Wales in recent years, but some of them are rarely seen and others, though not uncommon, are of little significance. Unfortunately, it is not possible to give figures of the actual losses caused by any of these diseases in terms of yields or in pounds, shillings and pence, for none are available, but this deficiency may ultimately be made good if those who are now endeavouring to devise standard methods of recording diseases quantitatively in the field are given the encouragement and help they deserve. On the other hand, the information gathered during the surveys organized by the Ministry of Agriculture and Fisheries since 1917 does enable one to form a fairly clear picture of the relative importance of the different cereal diseases, and that is all I shall attempt. My remarks refer only to England and Wales for, as you will hear from Dr Dennis, the position in Scotland is rather different. With few exceptions, the major diseases are found among the rusts, smuts, foot rots and stripe diseases, but none of them is responsible for the heavy economic losses caused by them from time to time in one or other of the main cereal-growing areas of the world.

Probably all the cereal rusts are heteroecious, but most of them survive the winter in the uredospore stage on self-sown cereals and autumn-sown cereal crops and, as a rule, it is the effect of winter conditions on the uredospore stage rather than the presence or absence of the alternate host that determines the relative severity of these fungi in England and Wales. Yellow rust (*Puccinia glumarum* (Schm.) Erikss. & Henn.) is the most important of them; it is worst on wheat, fairly common on barley and occasionally attacks rye. The alternate stage, if one occurs, is unknown, and the survival of the rust is dependent on the overwintering of the uredospores which, though often killed by high summer temperatures, can withstand many degrees of frost. Consequently, the rust gets a quick start in spring, and after mild winters when the uredospores survive in quantity, the attack may be early and heavy, as in 1943. After hard winters or very hot summers it is usually scarce. None the less, temperature is not the only important factor; last winter was a mild one, but yellow rust is nothing like as serious this year as after the equally mild but wetter winter of 1942–3. In fact, even though this is our most injurious rust of cereals, only about once in 10 years is it sufficiently bad to reduce yield by more than an estimated 5–10%. A combined attack of yellow rust and mildew will lead to many badly filled ears and shrivelled grain.

Black rust (*Puccinia graminis* Pers.) never causes such devastating losses as are experienced, for instance, in North America in favourable rust years, and on the whole it has very little economic significance here. The uredospores are generally killed by winter temperatures and, in most years, the source of the rust is affected barberries. It occurs every year on wheat in south-west Wales where barberry is common, and slight attacks may develop in a few other districts, but the reservoir of uredospores is slow in building up on the wheat plants, spread is slow and the rust usually comes late and does little

damage. Oats are not infrequently attacked but the rust is rare on barley and rye. Very occasionally, as in 1940, black rust becomes epidemic in many districts in August and then it affects yields. It is thought that these severe attacks arise in years when large numbers of uredospores are blown over from the Continent. The brown rusts of wheat, barley and rye are common and apparently harmless but crown rust of oats (*Puccinia coronata* Corda) leads to indifferently filled ears and poor yields in Wales and south-west England in years when the foliage becomes golden yellow with it in June or July.

Of the smut fungi, wheat bunt (*Tilletia caries* (DC.) Tul.) is still the most important and is still responsible for the loss or spoilage of thousands of bushels annually, despite the ease with which it can be prevented. Indeed, it begins to look as if history might repeat itself. For some years after the last war it was by no means rare in England and Wales to find 20–50% of the ears in a wheat crop bunted, and in 1921–2 one-third of the wheat samples examined at the Official Seed Testing Station, Cambridge, were visibly bunted. The following year the percentage of bunted samples was 16, after which the figure dropped slowly and more or less regularly until in 1940–1 it was as low as 1·2%. In the last two years there has been only a slight increase in the Cambridge figures but the number of badly bunted crops reported from other sources certainly suggests that bunt is getting more prevalent again. This is surprising in view of the much greater amount of seed dressing now carried out. Possibly the fact that more English wheat is used for milling purposes under war-time conditions has focused greater attention on bunt, but this can scarcely be the only explanation. It is generally thought that soil infection does not occur in this country but the point is one that may well repay investigation. Incidentally, as far as I am aware, there is no authentic record of the smooth spored species *Tilletia laevis* Kühn on growing crops in Britain, though it has been seen once on imported chicken corn.

The other cereal smuts are less important. Occasionally wheat crops may show 15% or more of the ears affected with loose smut (*Ustilago Tritici* (Pers.) Rostr.) but the great majority of crops are free from it or show less than 1% affected ears. Flag smut (*Urocystis Tritici* Koern.) is unknown in Britain. On barley, covered smut (*Ustilago Hordei* (Pers.) Lagerh.) is commoner than loose smut (*U. nuda* (Jens.) Rostr.) but is much less frequent now than it was 20 years ago and only occasionally ruins a crop. A third barley smut, *Ustilago nigra* Tapke, which is peculiar in that it is a loose smut which contaminates the seed grain without infecting it, has not yet been recognized here. It is not easy to distinguish the two smuts on oats and it is doubtful if they deserve to be regarded as more than varieties of the same species. Loose smut (*Ustilago Avenae* (Pers.) Jens.) is much more common than covered smut (*U. Kolleri* Wille) and, occasionally, attacks involving 10–30% of the ears are seen.

Of the two main foot rot diseases I shall say little as they will be dealt with later. As regards take-all (*Ophiobolus graminis* (Sacc.) Sacc.) we can combine pride in Mr Garrett's achievements with relief that the disease has proved to be less injurious here than was at one time feared. Some bad outbreaks have occurred where wheat or barley have been grown too often, but the disease is prominent and troublesome only in wet seasons and on light soils overlying chalk or limestone, where the rotation is too short. The form on oats (*O. graminis* var. *Avenae* E. M. Turner) is more or less restricted to those areas—Wales, the north-west and north—where oats is the chief cereal crop. Eyespot (*Cercosporella herpotrichoides* Fron) is probably much older than is suggested by the date of its recognition in England (1935) but the precise importance to be attached to it is a matter of opinion. The disease causes lodging in wheat as well as thinning out in spring, particularly in eastern districts, and if responsible for anything like the bulk of lodging that occurs, it is indeed a very serious disease. Perhaps Miss Glynne will be able to enlighten us on this point; for my own part I am perhaps unduly swayed by the fact that oats, which lodge freely, are highly resistant to eyespot.

In 1942 a rather severe attack of scab (*Gibberella Zeae* (Schw.) Petch) in mid-Wales indicated the possibilities of this hitherto rare disease, but brown foot rot (*Fusarium* spp.), though widely distributed, is harmful only in heavy, poorly drained, acid soils. Various other fungi may be associated with foot rot, but with the possible exception of species of *Leptosphaeria* they are unimportant. The apparent rarity of *Helminthosporium sativum* Pamm., King & Bakke in this country is surprising in view of its severity in some other countries.

We know very little of the effect on yield caused by leaf stripe of barley (*Helminthosporium gramineum* Rabenh.) and leaf spot of oats (*H. Avenae* Eidam). Both are common everywhere, as is confirmed by the high percentage of contaminated seed samples received at the Official Seed Testing Station, and both may be responsible for poor stands, especially in northern and western districts, but really serious losses seem to occur only in isolated instances.

Of the many other cereal diseases, I will mention only two—mildew (*Erysiphe graminis* DC.) and ergot (*Claviceps purpurea* (Fr.) Tul.). It is astonishing how little attention has been given to a fungus as common and well established as mildew. Practically nothing is known about its behaviour in this country or its effect on yield. A bad mildew year usually follows a mild winter and dry spring and under such conditions, especially where yellow rust is also severe, deaf ears and shrivelled grain may be expected. More concern is usually felt when the mildew becomes

conspicuous on the ears themselves but the adverse effect is probably much less marked than when the foliage is shrivelled by it earlier in the season. Ergot can generally be found in small quantities in English cereals; in descending order on rye, wheat, barley and oats, but the disease is not an important one here. Infection occurs through the open or nearly open flowers and moisture is necessary for the spores to germinate. Consequently, ergot becomes prominent only when cool, wet weather prolongs the normally short flowering period. During the past 25 years these conditions have been fulfilled only a few times, notably in 1927 and 1928, and in those years ergot was well above normal.

The other parasitic cereal diseases are at present local, rare or unimportant. Of the 50 or more diseases listed on cereals, therefore, the chief ones from the economic standpoint and taking the country as a whole are yellow rust, bunt of wheat, take-all, eyespot, leaf stripe of barley, leaf spot of oats and mildew. To these it may be necessary to add various deficiency diseases, but with the exception of grey leaf of oats (manganese deficiency) we are only just beginning to learn something about these troubles. It is not unlikely, however, that at the present time mineral deficiencies and imperfect cultivation play a greater part in determining poor yields than diseases caused by parasites.

Biologic specialization in the cereal rusts

By F. T. Brooks, *Botany School, Cambridge*

The rusts to be discussed are *Puccinia graminis*, *P. triticina*, *P. glumarum*, *P. anomala* and *P. coronata*. In this country *P. graminis* or stem rust occurs occasionally on wheat, barley, rye and oats, and also on certain grasses, e.g. *Agropyron repens*, but except in a few areas it usually attacks cereals so late in their growth if at all that little or no damage is done. In many seasons it is completely absent in East Anglia. This is very different from the devastating attacks recorded in the eighteenth and early part of the nineteenth century. In Britain this rust is chiefly seen on the stems, and it is rare for pustules to occur on the leaves. *P. triticina*, the brown or leaf rust of wheat, commonly occurs in epidemic form on the leaves in late June or early July, but rarely does appreciable harm here although more explicit information about this is desirable. In some parts of the United States and in Argentina serious losses may be caused by it. *P. glumarum*, yellow rust, is frequently epidemic on wheat, but is rarely seen to any great extent on barley in this country. It may occur abundantly on wheat as early as February, and its appearance in quantity is invariably earlier than *P. triticina*. It thrives at a lower temperature than *P. triticina* and *P. graminis*. Serious attacks of yellow rust may appreciably reduce yield through shrivelling of the grain, but many wheat varieties grown in this country are moderately or markedly resistant. It is the only wheat rust that British plant breeders have seriously taken into account. *P. anomala*, the brown or dwarf rust of barley, occurs commonly, but epidemic attack is usually late here and little harm is done. *P. coronata*, the crown rust of oats, is not infrequent in the west and north where it sometimes reduces yield. On the whole we are fortunate in that cereal rusts do so little harm in this country and that we have not to face the disastrous attacks which sometimes occur in other countries.

Each one of these rusts comprises a number of smaller units, varieties and/or physiologic races, having diverse host relationships, but differing only slightly or oftener not at all in morphology, a condition emphasized by Eriksson in 1894. It is indeed in the rust fungi that the phenomenon of biologic specialization is best exemplified and in which the most intensive study of host relationships has been made. Those who have not themselves taken part in the delimitation of these races are not unnaturally bewildered by, and perhaps somewhat sceptical of, their multiplicity, but there is no doubt that these diverse races are definite entities. After all, this phenomenon is only an illustration of the multitude of units in Nature below the rank of a species, but here expressed almost exclusively in physiological terms. From the economic standpoint precise knowledge of the different physiologic races is important so that plant breeders can take them into account in synthesizing resistant varieties.

The races of each of these rusts are determined by their behaviour on a limited number of differential host varieties in the seedling stage grown under constant environmental conditions, these varieties being selected from a larger number tried out in preliminary experiments as being the most suitable for the purpose. The races are designated by numbers.

With regard to *Puccinia graminis*, it is customary first to divide it into a number of varieties, e.g. *P. gr. tritici* on wheat but affecting also certain barley varieties, *P. gr. avenae* on oats and certain grasses, *P. gr. secalis* on rye, some barley varieties, and certain grasses including *Agropyron repens*, and a few other varieties chiefly confined to grasses. The uredospores of these varieties show slight but constant differences in size. Within each of these varieties of *P. graminis* there are a number of physiologic races.

Within *P. gr. tritici* nearly 200 races have been defined by their infectivity of the differential host varieties, chiefly by Stakman and his colleagues in the United States. Many occur in North America and a considerable number have been found in Australia, India, Kenya Colony, South Africa, South America, Germany and other parts of the world. On the other hand there is no knowledge of the particular races of *P. gr. tritici* which occur in Britain, but this is largely only a matter of academic interest in view of its comparative rarity. In Germany, Hassebrauk (1939) found eleven races of *P. gr. tritici* in 1937. It is a somewhat staggering thought that there are nearly 200 races of *P. gr. tritici.* Many have been shown to be heterozygous, and selfing and hybridization experiments with the stage on the barberry have clearly established that new races may arise in connexion with the formation of aecidiospores. There is evidence too that genic mutation, involving changes in pathogenicity, may occur in the uredospore stage. It must not be supposed that all these races are equally abundant. In Canada, the United States and other important wheat-growing countries a few races usually predominate in any one season, though the particular races may differ from year to year. Stakman, Loegering, Cassell & Hines (1943) record that in the first half of the decade 1930–9 races 36, 38 and 49 were the most important in the United States whereas race 56 was by far the most abundant during the second half; since 1939 races 56, 17 and 38 have been predominant there. As already stated the races are determined by their reactions on seedlings of the differential varieties. Now it has been shown in North America, first by Goulden, Neatby & Welsh (1928), that a wheat variety susceptible in the seedling condition to a particular race may be resistant to it in the adult state. This discovery of adult or mature plant resistance is of the greatest importance to the plant breeder for it has been shown to concern many physiologic races of the rust. With regard to *P. gr. avenae* an analysis of the races has been made in North America, Germany and some other countries, but the number so far determined is only thirteen according to Stakman & Loegering (1944). These authors call attention to the recent increase of race 8 in the United States on varieties of oats bred for their resistance to other races.

More than 100 physiologic races of *Puccinia triticina* have been defined, chiefly in North America, the host varieties used for determination being different from those used in connexion with *P. gr. tritici.* The investigations of Miss F. M. Roberts (1936) provide information about the races which occur in this country. She examined forty-three collections of this rust from different parts of England and Wales and determined the existence of ten races, nos. 10 and 15 previously recorded, and nos. 66–73 which were then new. In 1931 and 1932 only one race was found at Cambridge but in 1933 four races were recorded. It is somewhat remarkable that eight of the races were first discovered in this country in view of the apparent absence of the aecidial stage on *Thalictrum* here. This may perhaps point to genic mutation in the uredospore stage being an important factor in the evolution of new races, some evidence for which was indeed found by Miss Roberts in the course of her work. Hassebrauk (1937) found 14 races in Germany and neighbouring countries during 1934–5. In a more recent paper (1940) he has shown that some races of *P. triticina* are really composite groups, for by choosing a new and more extensive range of differential hosts he established the existence of no less than forty-five new races. He points out that the differential varieties most suitable for diagnosing the races in one country are not necessarily the best for another country, and concludes that biologic specialization in *P. triticina* is so complex that it may be unprofitable to study it further. On the other hand a multitude of physiologic races does not invalidate an unexpectedly wide host relationship for some of them. Johnston (1940) states that some species of *Agropyron* are susceptible to certain races of *P. triticina* under experimental conditions, and I have found that seedlings of *Bromus asper* are susceptible to one race of this fungus. The phenomenon of adult plant resistance is also exhibited towards races of *P. triticina,* as pointed out by Newton & Johnson (1943) with regard to the varieties Regent and Renown in Canada.

Much of the work on the determination of the races of *Puccinia glumarum* has been done in Germany, chiefly by Straib, who received collections from several countries as well as from many parts of Germany. The differential varieties were selected in Germany and not in the United States as for *P. graminis* and *P. triticina.* Up to 1939 Straib (1939) had delimited forty-seven races, at least twenty of which occur in Germany. Unlike Eriksson, who divided *P. glumarum* into *P. gl. tritici, P. gl. hordei, P. gl. secalis,* etc., i.e. into a number of varieties as they would be called now, Straib and other German investigators found that some races infected equally well some varieties of wheat, barley, rye, and also certain grasses. This has been confirmed by Newton & Johnson (1936) in Canada, hence Eriksson's nomenclature for this rust has been abandoned. There is little information about the races of *P. glumarum* which occur in Britain. During 1931–4 Miss M. Cudmore (now Mrs Bawden) examined at Cambridge many collections of yellow rust on wheat from different parts of the country and showed in a preliminary way that several races were involved. Her work, however, was interrupted and the results were never published. In 1931 and again in 1932 single collections of yellow rust on wheat in England were determined by Newton & Johnson (1936), working in Canada, as race no. 4. Yellow rust on

wheat at Cambridge sent to Straib in Germany by Dr S. Dickinson in 1933 proved to be race no. 4 and another collection sent in 1934 was race no. 6. Mrs Bawden's preliminary results showed that more than two races occur in Britain, and it is desirable for a comprehensive survey of the races of yellow rust to be made in this country after the war so that the relative susceptibility of the common wheat varieties to individual races can be determined. In 1943, when there was a considerable epidemic of yellow rust, the variety Desprez 80 was badly attacked in the south but was often only slightly affected in the north, a result most easily explained by the occurrence of different races of rust in these regions. Further information about the susceptibility of wheat varieties to yellow rust in different parts of Britain is required, and the scheme of Dr F. T. Bennett and other advisory mycologists to make observations thereon during 1944 is to be welcomed. Such observations should be extended over several years as yellow rust varies greatly in incidence from season to season. In determining the races of yellow rust a special difficulty is encountered, for unless precautions are taken the cultures are liable to be lost during hot weather. Theoretically, having established single-spore cultures of a particular collection of rust, one series of tests on the differential varieties should be sufficient to determine the race number or numbers of the collection. It is preferable, however, and in my opinion essential, for several series of tests to be made before conclusions are drawn so that it is often necessary to maintain the cultures for more than a year. Again it may be asked, how have the many races of *P. glumarum* come into existence? The only suggestion of an aecidial stage I know of is one made by Tranzschel (1934) who thought that an aecidium on species of *Valerianella* in the Crimea and Caucasus might be a stage in the life cycle. As far as I am aware this surmise has not been substantiated. It seems certain that no aecidial stage occurs in western Europe, and it is likely therefore that some of these races have arisen by gene mutation. In referring to *P. graminis tritici* mention was made of the phenomenon of mature plant resistance. Whether this occurs also in relation to *P. glumarum* is uncertain, but one observation certainly points towards it: Little Joss wheat is occasionally heavily infected with yellow rust in February, but, even so, it becomes markedly free from this rust as maturity approaches.

The physiologic races of *Puccinia anomala*, the brown rust of barley, have been studied by Mains (1930) in the United States, Brown (1931) in Canada, Hey (1932), Ronsdorf (1935) and Straib (1937*a*) in Germany, and by d'Oliveira (1939) in Britain and Portugal. Separate sets of differential host varieties have been used by investigators in North America and Germany, but an attempt at standardization has been made by d'Oliveira (1939). At least thirty races have been identified of which four were first discovered in Britain, one being widely distributed here. d'Oliveira describes the occurrence of a mutant in spore colour and pathogenicity among his cultures, and also the origin of apparently new races through the aecidial stage on *Ornithogalum umbellatum* in Portugal. He also records the presence of slightly different biotypes within certain races, i.e. minor forms showing small but apparently constant differences on some of the standard varieties. Biotypes have also been described within specific races of other cereal rusts, notably in *P. graminis tritici*, and it is difficult to know where to draw the line between a biotype and a physiologic race. Again it must be emphasized that if the range of differential hosts is extended new biotypes or even races may be discovered which could not be detected with a narrower range of host varieties.

As with *Puccinia graminis*, it is customary to divide *P. coronata* into a number of varieties, one of which, *P. cor. avenae*, is the crown rust of oats, with its aecidial stage on *Rhamnus cathartica*. Another is *P. cor. lolii*, which is so abundant in certain seasons. *P. cor. avenae* may also infect certain grasses, and *P. cor. lolii* may slightly infect some oat varieties. Much attention has been paid in North America and Germany to the physiologic races of *P. cor. avenae*, but distinct series of differential varieties have been used by the chief investigators, Murphy (1930) in the United States, Frenzel (1931) and Straib (1937*b*) in Germany, so that the nomenclature of the races has not yet been adequately stabilized. It is clear, however, that many races occur both in North America and in Germany. Indeed, Straib (1937*b*) claims to have identified no less than 139 races from 144 collections of rust on *Avena sativa* and *A. fatua* in Germany. In her work on *Puccinia coronata*, Miss M. R. Brown (1937) (now Mrs Gilson) paid some attention to the races of oat crown rust occurring in Britain. She found three, one which had been previously described in North America and Australia and two which were then new. Since then she has found four other races in Britain, determined by means of Murphy's differential varieties (1930). With *P. cor. avenae* also it can be shown that certain races are really composite groups. For instance, Kingsolver & Murphy (1940) found that twenty-four isolates, all of which belonged to race no. 1 according to tests on Murphy's differential varieties (1930), could be separated into eighteen races by tests on Straib's differentials (1937*b*).

Attention may now be called to some of the difficulties encountered in determining the physiologic races of these rusts. Genetic purity of the differential varieties and of the rust cultures is of course essential. Seed stocks of some of the varieties received from another country may occasionally be impure and it may take one or more seasons to eliminate rogues. Fortunately, the cereals are chiefly self-pollinating so

that once a pure stock has been obtained the risk of out-pollination is small especially if the ears are bagged before flowering. The only certain method of obtaining a pure rust culture is to establish it from a single uredospore, for more than one race may occur on a leaf gathered in the field. The pure cultures must be kept in isolation from each other, e.g. in cellophane cylinders or cages, at a temperature which the rust tolerates. With *P. glumarum* it may be necessary to store the spores at a low temperature before hot weather intervenes. The spores of these rusts remain viable for several weeks if kept at a suitable temperature and humidity, and storage in this way may obviate renewal of the stock cultures during busy periods. The investigator must always be on the alert against contamination of his pure cultures by extraneous races. Few glasshouses can be kept entirely free from insects and there is always risk of the transference of spores when the stock cultures are exposed. The host varieties are inoculated at the same stage of seedling development, usually on the first or second leaf, and are covered by bell-jars for 48 hr. in order to facilitate infection. The reactions should be studied under environmental conditions as constant as possible. The soil should always be of the same kind, for differences in content of nitrogen and other constituents influence susceptibility. The other most important environmental factors are temperature and light intensity. Each rust has an optimum temperature for development, e.g. 22–25° C. for *P. graminis* and 13–16° C. for *P. glumarum*, and at some centres the tests are made in temperature-controlled glasshouses. With temperatures appreciably higher than the optimum the differential hosts usually become more resistant. Where temperature control is not available the tests can be carried out at varying periods during spring, summer and autumn when the range of temperature approximates to that of the optimum for the particular rust. It is more difficult to control light intensity, but the tests must be made when the light is good. At Cambridge it is useless to test physiologic races during the winter because of long spells of very dull weather. Lastly, it is preferable to test simultaneously as many races of one rust as possible in order to eliminate environmental differences; this is especially important where there is no rigid temperature control.

In conclusion, perhaps one may be permitted to refer to one's personal outlook on the results achieved in the determination of the races of cereal rusts. From the academic standpoint they are of great interest if somewhat bewildering. Each of these rusts has been shown to exist in a very large number of distinct physiologic entities, separated sometimes by large, sometimes by small, differences. One is reminded of a somewhat comparable phenomenon in *Cladosporium herbarum* in which isolations from natural substrata are rarely identical in culture, the differences here, however, being of a morphological kind. In view of the extreme biological specialization in the cereal rusts it is doubtful whether further study along these lines will be profitable except in particular instances, especially in relation to plant breeding programmes. This work on biologic specialization has also made it abundantly clear that the rusts are still liable to change by hybridization and genic mutation. From the plant breeder's standpoint the discovery of physiologic differentiation in the cereal rusts is of paramount importance and has had a profound influence on his work. He need not concern himself with all the innumerable races that have been described, because, as has been pointed out by Neatby (1942) and others, many of them behave as single groups in the field. Furthermore, the discovery of mature plant resistance to many races has greatly alleviated the situation. The breeder must, however, maintain a watchful outlook, for a new virulent race may develop at any time. The position can only be safeguarded by continued co-operation between pathologists and plant breeders.

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Looking back and looking forward. (A retrospect of cereal diseases in East Anglia in the past 21 years.)

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For the past 21 years observations have been made on cereal diseases in East Anglia, and it may not be inappropriate now to review briefly their prevalence and intensity during this period. In the course of this survey, which spans the years between 1923–44, we shall take the opportunity of making certain observations, particularly as they apply to seed-borne diseases and their control by the appropriate methods of seed disinfection.

In 1923, the majority of farmers used copper sulphate to disinfect their seed wheat against bunt, but some, a small number only, used formalin. Both chemicals were effective fungicides, but the phytocidal effect of copper sulphate was greater, as repeated trials showed that it usually reduced germination. For this reason, therefore, and also because it was the more effective fungicide, the use of formalin was advocated. With both these wet treatments, however, it was necessary to dry the grain quickly and to sow as soon as possible, otherwise germination was impaired. As might be expected this difficulty was overcome by employing powders as seed disinfectants, and one of the first to be used was a basic copper carbonate. This was used with success in Australia by Darnell-Smith, and later was tried out by research workers in this and other countries. It was tested on the trial plots on the Cambridge University Farm in 1924–6 and was found to be satisfactory for wheat provided this was not too heavily contaminated with bunt spores. In 1925, because it was considered that there were definite advantages in dusting seed grain in this way, a machine designed for this purpose was imported from the United States. It was power driven and was equipped with a suction fan to reclaim any excessive dust and to prevent this from escaping into the surrounding air. It worked well, and was subsequently used by the Director of the Cambridge University Farm, Mr A. Amos, to dress seed wheat for his clients if they required such grain to be disinfected. As the variety which we grew in those days was largely Little Joss, it will be realized that the farmers who bought this seed were assured of protection from two diseases, namely yellow rust and bunt, for this light-land wheat, which had been raised by Professor Biffen, has resistance in its mature stage to *Puccinia glumarum*.

The introduction of this machine, and its subsequent use, is the first record in this country of such a farm implement, and it is here recorded because, together with the experimental work which was being done at that time by the seed firm of Messrs. Gartons, it represented a practical endeavour to limit the spread of disease by the disinfection of seed

stocks before they were received by the farmer. As much is often said of the high depreciation that occurs with farm machinery, it is of interest to say that we still use this dusting machine and, as our crops are not affected with bunt of wheat, leaf spot and smut of oats or covered smut and leaf stripe of barley, we assume that it is still effective. But perhaps our freedom from these diseases is due most to the dry dressing which we now use, for we ceased using copper carbonate in 1931. But here a digression is necessary. In the 1920's a good crop of spring oats seemed to depend upon the whims of providence, as in a sense I suppose it did. The plant pathologist, materialistically minded, looked for the causes of these failures which so frequently occurred. He found them in the ravages made by frit fly and leaf spot disease. It was not long, however, before entomologists were demonstrating that frit fly attacks could be circumvented by sowing spring oats early, but what could the mycologist do to lessen the attacks made by *Helminthosporium Avenae*? At that time many thought that a few odd spots, or stripes, on oat leaves were of little or no account, but unfortunately they overlooked or were unacquainted with the more sinister phase of this disease, namely *pre-emergence blight*. The importance of the latter is well indicated by an experiment made on the Cambridge University Farm in 1925 (Weston & Taylor, 1943). A detailed field analysis was made of a high germinating sample of Black Supreme seed oat known to be infected with *Helminthosporium Avenae*. 52% of the seedlings appearing above ground were affected with leaf spot, but 5% only died—not a very serious mortality. But what happened in the soil? 56% of the total seed sown was killed before emergence, and the evidence indicated that the cause was *Helminthosporium Avenae*. At that time, however, such failures were often attributed to wireworm, cold winds, or frost injury. Then, as now, it was thought by many (unjustifiably, I think) that Scotch seed was more liable to be affected. But what advice on control measures was the practising plant pathologist to give? He could suggest a change of seed, and hope that if the advice was followed, the new sample would be one free from infection—but that was all he could do. Treatment of the seed with formalin was of little or no avail, and copper sulphate, although giving some measure of control experimentally, could not be used in practice as it markedly reduced germination. Copper carbonate was not effective and, moreover, it was not in general use for only relatively few farmers used it for dressing their wheat to prevent bunt.

Running parallel, as it were, with this disease of oats was the somewhat similar seed-borne disease, leaf stripe of barley. Here again no effective seed dressing was available although some control was claimed by the use of formalin. It will be realized therefore that the introduction in 1929–30 of a dry seed dressing which gave control not only of these diseases but also certain smut diseases as well, was an event of outstanding importance. Now had I been reading this paper then I should have referred to this dressing as a trade product thought to contain 1·5% of mercury in the form of phenyl mercury acetate. Reading it in June 1944, I refer to it simply as *Ceresan*. But why this departure from our strict professional etiquette? Is it not because Ceresan is now a seed dressing officially approved by the Ministry of Agriculture and Fisheries? Have its merits not been assessed by a body of experts assembled together in a committee room? Is it of consequence therefore to you now, or to me, or to the farmer, to know what it contains? As this question raises matters of some importance let us leave it to mature for a while, and continue with our survey through the cornfields.

In the depressing 1930's, the lot of an Advisory Mycologist was not a happy one. Times were bad. Take-all was rampant on many a farm, and why? Money had to be found to pay men worthy of their hire—cash crops were necessary, and consequently the land was corned too heavily. The soil became sick with *Ophiobolus*, just as a soil may become sick with *Sclerotinia trifoliorum* or with *Heterodera schachtii*. To-day take-all cannot be considered a major problem although, of course, it sometimes presents difficulties on the lighter-textured soils, and in cereals which follow broken up old grassland. It does not occur to any appreciable extent under a good system of crop husbandry—namely, on soil in good heart, free from weedy rubbish, and under a sound rotation. If a crop of wheat or barley has shown whiteheads we advise the farmer not to follow with either of these crops, and this applies particularly to the farms on the lighter lands. If a corn crop is imperative then we suggest that oats should be taken, as in East Anglia we have no evidence as yet of the presence of *Ophiobolus graminis* var. *Avenae*. Until recently we have considered a clover and rye grass ley as a potent source of danger in assisting the fungus to carry over, and then to multiply on the roots of more susceptible crops. Recent work by Buddin & Garrett (1944) indicates that this danger may not be as great as it is thought to be. Truly the 1930's were depressing, but relieved scenically perhaps by flaming fields of poppies and the swansdown of thistles. Is that an overstatement, perhaps it is, but I make it to draw attention to a matter which some of you may not appreciate, namely the remarkable cleanness of our land to-day. That too is helping disease control and very markedly.

But what of root rot of cereals, and in what degree do they affect our crops? *Fusarium* spp. are not infrequently associated with thin stands, and deaf or partially deaf ears, but when this happens, and the attack is a severe one, it can usually be correlated

with abnormal soil conditions. On land in good heart, well drained, and in a state of high fertility, it is unusual to find failures which can be attributed directly to the presence of these organisms.

Weston & Garrett (1943) have noted that in Norfolk, *Rhizoctonia solani* is sometimes associated with a root rot of cereals, but the disease is of minor importance. In the spring, patches of backward and stunted plants become noticeable in affected crops; these patches are usually small and sharply defined. Affected plants usually die by mid-June, but others which survive are much delayed in growth. It is not unlikely that this disease may also be associated with certain localized abnormal soil conditions. But this is a very small matter compared with another problem we are investigating. On many thousands of acres of reclaimed fenland, good root crops can be grown but not good cereal crops. The corn comes away well, but yields at harvest are disappointing. In the past two or three years these crops have been smothered with mildew, and it has been thought by some that this is the cause of the partial failures. The problem, however, is probably a complex one involving many factors, and research on these is in progress.

But perhaps the time is not too far distant when the plant breeders will consider it opportune to produce a stiff-strawed, mildew, rust and 'eye-spot lodging' resistant wheat!

Continuing our journey through the cornfields we come across a crop of diseased barley. Will the leaf blotch on it affect the yield or, what is more important, its quality as a malting sample? We have no evidence at all on this matter. In some years, but infrequently, the disease is very noticeable and then it is that we receive complaints that these modern seed dressings are not nearly so effective as they are claimed to be, and farmers write to their agents about it, and their agents write to us, and we write to the farmer explaining the position. Namely, that the leaf blotch fungus, *Rhynchosporium secalis*, is not seed-borne, and consequently the use of an organo-mercury seed dressing cannot be expected to control it. The disease is usually more marked in a wet May, and it appears to carry over from year to year on volunteer barley plants, but apart from barley and rye it does attack a number of grasses such as cocksfoot, wall barley, couch and soft and sterile brome. With the exception of work done by Brooks (1928) this disease has received little attention in this country, and that is why we are at present re-investigating it.

And the rust diseases—what of these? Normally they are not very serious although on farms near the east coast susceptible varieties are often severely attacked. The resistance and susceptibility of wheat varieties to yellow rust vary from complete resistance to extreme susceptibility. It does not follow, however, that a variety which appears to be resistant in one part of the country will be resistant in another. This is because the rust fungus exists in specialized forms or strains, and these are not necessarily uniformly distributed. It is only by observations carried out over a number of years that the relative resistance of varieties to rust attack can be assessed. The resistance of Rivet wheat is so strong that it could almost be described as immune, and Little Joss, once it has passed the seedling stage, is markedly resistant. On the other hand, exceptionally severe attacks have been encountered on Desprez 80. Between these extremes fall the remainder of the commonly grown varieties, which differ in their degree of susceptibility.

Dr H. Hunter, of the National Institute of Agricultural Botany, informed me that: 'Judging varieties of wheat from our experience here and at various centres throughout the country, the following is a list in order of value, based on resistance to yellow rust: Rivet, Little Joss, (Steadfast, Holdfast, Yeoman), Jubilegem, (Bersée, Juliana), Scandia, Als, (Squarehead's Master, Wilhelmina, Victor), Wilma, Desprez 80. The varieties listed together in any one set of brackets are equal or very similar to one another in their resistance to yellow rust. This list may or may not require slight adjustment according to locality....' Apart, however, from any question of susceptibility to disease, the wheat variety should be chosen primarily to suit the soil texture and the standards of fertility.

Brown rust develops later in the season and the losses caused by it are seldom severe. Black rust is rare and when present is usually noted during the last week in July or the first week in August.

But here let us leave our survey for a while and ponder some of the perplexities of the early 1930's. Did it matter what Ceresan contained? Perhaps a medical man might have asked, 'Does it matter what Prontosil is?' Was it of particular interest when—in the strictest confidence—chemists of some learning informed you that Ceresan was thought to contain 1·5% mercury in the form of phenyl mercury acetate? Surely here was a challenge not only to trade but also to academic research. Needless to say that challenge was accepted, but independently. Agrosan competed with Ceresan, and Weston & Booer (1935) came to the conclusion that in the series $R.\mathrm{Hg}.X$ where R is a hydrocarbon and X an acidic radical, the fungicidal power decreased with increase of the molecular weight of R. At that time was it not immaterial which was the better, provided, of course, the difference was relatively slight. Competition is the life blood of trade—it may be ruthless; but through it good accrues because eventually it eliminates—*and will eliminate*—the less worthy. Let the years flow on then so that we may see how this matter works out in practice.

Here, with an Agricultural Organizer, we are contemplating a field of oats, and the crop is badly smutted. Indeed, in this district at that time, oat smut was prevalent and intense in most oat crops. To-day it is less evident. But that colleague of ours

requires answers to the following questions which are these:

Q. Can loose and covered smut of oats be prevented?

R. Yes.

Q. What is the most effective fungicidal treatment?

R. The wet treatment, formalin.

Q. Do you recommend this?

R. In the majority of cases, no. The organo-mercury seed dressings are easier to apply, there is less risk of injury to germination, and for the most part they give a very fair control of these diseases.

Q. What are the organo-mercury seed dressings, at present on the market, that claim to give an effective control of these diseases?

R. Ceresan, Agrosan, Lunasan, Abavit, Leytosan and Harvesan.

Q. Which are the best of them?

R. The best products are those which give the best disease control with the least possibility of phytocidal effect, and which are relatively safe to handle and are reasonably fool proof.

It may seem to some that this is an evasive reply. To an Agricultural Organizer, of course, it would be —but I have inserted it here, with due deliberation, for the attention of the trade. I need hardly say that those organizers, or farmers, who consult us on such a matter get a straightforward answer, and this we base on the results obtained from our trial plots. *The intrinsic test of a seed dressing is its performance in the field.*

Are we justified in giving a straightforward reply? Let us examine the results of the seed disinfection trials carried out on the Cambridge University Farm in 1942, so that we may see how Abavit, Agrosan, Ceresan, Harvesan, Leytosan and Lunasan behave in preventing leaf stripe of barley, leaf spot of oats, and bunt of wheat. As a precautionary measure, however, let us randomize these products and designate them by the letters A, B, C, D, E and F.

First the barley. The percentage of barley seedlings showing leaf stripe from treated and untreated plots is as follows:

Treatment	Percentage of leaf stripe
A	0·27
B	0·00
C	0·18
D	0·00
E	0·38
F	0·19
Untreated	34·36

Now to oats.

Treatment	Percentage of leaf spot
A	10·30
B	4·26
C	6·78
D	0·37
E	7·25
F	0·91
Untreated	23·94

Lastly, the wheat.

Treatment	Percentage of bunt
A	0·05
B	0·00
C	0·00
D	0·00
E	0·04
F	0·00
Untreated	2·62

As these plots were randomized, replicated and the results carefully recorded, it is clear that these treatments are a class of seed dressings which give a very fair control of seed-borne diseases. *It must be noted, however, that some are better than others.* Further, that whereas leaf stripe of barley and bunt of wheat are very amenable to these preventive measures, leaf spot of oats is more difficult to control.

But suppose that we are again asked the direct question—which is the best? Can we answer this publicly? Is it not clear that this is a matter fraught with many complications to the farmer, the Adviser and to the trade. Perhaps it was on this account that the Ministry of Agriculture and Fisheries and the Department of Agriculture for Scotland started in 1943 a voluntary scheme for the approval of insecticides and fungicides marketed under proprietary brand names for the control of plant pests and diseases.

A scheme of this nature is excellent in its conception for it is designed to limit the spread of disease by insuring the use of reliable products only. Furthermore, it informs the farmer as to these products and, indirectly, it is a protection against quack remedies. It does not tell him, however, which are the best products—in fact it may mislead him because he is given a wide range of materials from which to choose and these vary in their relative effectiveness.

Perhaps at this stage we should bear in mind that the intrinsic test of a seed dressing is its performance in the field. May it not happen that a farmer might use an approved product which is at a lower range of effectiveness in the control of certain diseases, and obtain results not eminently satisfactory. Should this happen frequently the approval scheme would be brought into disrepute. But let us examine results from our test plots in 1943 to see if there is any justification for this outlook.

Wheat

Treatment	Percentage of bunt
A	2·31
B	0·26
C	0·75
D	0·81
E	0·73
F	5·51
Untreated	50·37

What is the criterion of reliability and approval? Is a reduction of disease from 50 to 5 % satisfactory?

Obviously a reduction to 2% is better, and anything below 1% better still.

But what control can we obtain in 1944 with A, B, C, D, E and F, for these are the dressings which we will use. The farmer can use Abavit, Agrosan, Ceresan, Harvesan, Leytosan, Lunasan, Swains' 'Triangle' Brand Organo-Mercury seed dressing, or Leytosan C.C. In fact he can—and will —use what he chooses.

Leaf spot of oats is a disease which it is very desirable to control; let us see how A, B, C, D, E and F behave:

Treatment	Percentage of leaf spot
A	9·15
B	0·36
C	9·18
D	0·00
E	8·73
F	0·00
Untreated	13·72

It will be noted that the control of this disease given by B, D and F is virtually the same, and an excellent one. The control given by A, C and E, on the other hand, is not nearly so good. It is not necessary here, however, for us to discuss why this should be, for many a false inference can be drawn. One thing, however, must be said. If the approval scheme is to maintain its prestige then: (*a*) the standard of efficiency must be very jealously guarded, and (*b*) that standard must be assessed by field tests and the results obtained made available to the farmer. It is clear that this would necessitate an Official Testing Station.

The writer gratefully acknowledges the assistance of Dr R. Eric Taylor in the tests made from 1941–44, and he is also indebted to Mr A. R. Loveless for additional assistance in 1944.

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Cereal diseases in Scotland

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In Scotland the oat crop is still the most important cereal and takes a far more prominent place in the agricultural system than in most other parts of Great Britain. The 1937 oat acreage was 818,666, or about 27·4% of the total arable area, then almost 3,000,000 acres. The corresponding percentage in England was about 13.

Over most of Scotland the most important oat disease is still the primary or seedling blight phase of leaf stripe, caused by the fungus *Pyrenophora avenae*. So much has been said and written about this disease in the past 15 years that I do not propose to discuss it in detail to-day. To show that the fungus is as prevalent as ever on Scottish seed oats I will quote the results of examination of the first 260 samples of oats received for the routine germination test at the Scottish Department of Agriculture's Seed Testing Station in the autumn of 1942. These represented a random sample of that year's seed, derived from farms scattered all over Scotland, without any attempt being made to select diseased samples. Moreover, as they were from the first consignments of 1942 they must have been drawn from the earliest harvested grain, which is likely to carry less infection than grain from crops harvested late and exposed for a long time to unfavourable weather. The amount of leaf stripe present was estimated by growing the seedlings in sand to the second leaf stage and then adding together the numbers showing striping of leaves and those with pre-emergence blight. The highest degree of infection found was 42%, the average was 11·5%, while only 41% of the samples contained as little as 5% or less of the grain infected with *Pyrenophora avenae*.

With such a high probability of loss from pre-emergence blight it is not surprising that Scottish farmers, especially in the wetter and higher lying districts, still pin their faith to organo-mercury seed dressings for the oat crop. I would like to take this opportunity of paying a tribute to the late Dr D. G. O'Brien, through whose work the use of dry seed dressings was introduced into Scotland. Though too deeply imbued with the Celtic temperament to be strictly scientific in his methods, O'Brien was the ideal local adviser, in the sense of a propagandist skilled in the art of getting new ideas across to

* Now at The Herbarium, Royal Botanic Gardens, Kew, Surrey.

farmers. As so much interest has been taken of late years in this somewhat difficult process it may not be out of place to consider briefly how it was done. Though born in England, O'Brien had the advantage of being a product of the local University and Agricultural College. He was thoroughly familiar with local farming practice as a result of his experience as a County Organizer in Dumbartonshire and was perfectly at home with the local dialect. Moreover, he had secured for his sole use a field on the West of Scotland Agricultural College farm where he could lay out demonstration plots on a field scale. These were big enough to be appreciated by farmers and he was able to explain them personally to the numerous parties of farmers who visited Auchincruive each summer. Finally, once he was satisfied that in Ceresan he had a seed dressing that would control leaf stripe he was able to persuade a local seed firm that there was money in it if properly handled. It was this realistic approach in securing the co-operation of local commercial enterprise which put seed dressing on its feet in the west of Scotland. An old established seed firm has intimate contact with the farming community and can sell a product far more readily than a flood of written propaganda, advertisements and circulars. The result was that in 1932, only 18 months after the first published mention of dry seed dressing in the Scottish Department's *Journal*, 266,795 lb. of Ceresan were sold in Scotland, sufficient to dress 2,134,360 bush. of grain. It may be accepted that almost the whole of this was used on oats. Oats seeding rates vary considerably according to variety, district and method of sowing but assuming an average rate of 6 bush./acre this treated grain would sow 355,727 acres or 41 % of the 1932 oat acreage. The advocates of seed dressing consistently stressed that a reduction in seeding to the extent of 1–1½ bush./acre could safely be made with treated grain. It is doubtful how far this was carried out in practice by farmers but in any case the assumption of an average seeding rate of 6 bush./acre probably errs on the heavy side, so that we shall not be far wrong in saying that half the oats sown in Scotland in 1932 were dressed with Ceresan. By the spring of 1933 five Scottish implement firms were advertising machines for applying dry disinfectants to oat seed. These varied from the simple barrel type to more complicated machines with automatic feed and their prices varied correspondingly from 70*s.* to £10.

Reference to the files of the *Scottish Farmer* shows that Ceresan was first advertised on 31 Jan. 1931, Agrosan G on 14 Jan. 1933 and Leytosan CC on 25 Feb. 1933. Harvesan appeared on the Scottish market at about the same time as in England. Abavit B was on sale in the autumn of 1931 but was not very energetically publicized. Thus, from 1933 onwards there have been at least four equally effective seed dressings available to the Scottish growers and it is impossible to give precise figures for total sales subsequent to 1932. There is every reason to believe, however, that their use continues at a satisfactory level. The practice among seed merchants of dressing seed oats before supplying them to their customers is also now well established in Scotland.

While the use of seed dressings is general in the west and to some extent in the north, there is considerable evidence to show that on the best Lothian soils and in other favoured districts of the east coast little benefit is derived from their use, even with heavily infected grain. Thus, my attention was drawn last year to an extensive field trial with Marvellous oat on a farm adjacent to the Seed Testing Station, Corstorphine. The laboratory test had shown the seed to be infected with *Pyrenophora avenae* to the extent of 42 % and with *Fusarium* spp. to 21 %, yet comparison of the brairds on plots sown with dressed and undressed seed showed only 20 % less plants from the undressed seed: 10 % of the seedlings from the undressed seed bore leaf stripe lesions on their first leaves and under less favourable soil conditions these would have perished from pre-emergence blight. Similarly on the Department's own farm Victory oats bearing 13 % infection with *Pyrenophora avenae* in the laboratory test, gave an excellent braird in the field, in which 14 % of the plants had leaf stripe lesions on the first leaf. Here, apparently, there had been no pre-emergence blight.

At this stage I should mention that although attention has been concentrated mainly on *P. avenae*, some species of *Fusarium*, especially *F. nivale*, are apparently also partly responsible for low brairds of oats sown under poor soil conditions. The samples of the 1942 seed crop, already referred to, showed on an average 21 % of *Fusarium* foot rot in the laboratory tests and in some samples this seed-borne disease attacked 80 % of the seedlings. *F. culmorum* and *F. nivale* were isolated from foot-rotted seedlings and small scale infection experiments were undertaken to investigate their pathogenicity to brairding oats. In the laboratory, at a temperature of about 14° C., strains of *F. culmorum* obtained from oats and from rotten potatoes proved actively pathogenic to oat seedlings in soil cultures and still more so in sand cultures. In soil they reduced the braird from 70 to 50 and 52 % respectively. *F. nivale* had a very similar effect in the laboratory. Out of doors, with inoculated seed sown in the ground on 24 Feb. at a soil temperature of 3° C., *F. culmorum* had little effect but *F. nivale* still reduced the braird from 66 to 40 %.

Gibberella zeae has been rather prevalent of late years on ripening oats and especially on straw injured by take-all or by insects in the south-western counties and in the islands. It was therefore also included in these inoculation experiments. It had no effect whatever on seedling growth or emergence. Equally negative results were obtained in a laboratory ex-

periment with a strain of *Fusarium poae* isolated from weathered and discoloured oats sent us from Aberdeenshire.

In addition to the injury caused by seed-borne *Fusarium* spores there is reason to believe that in the west of Scotland and probably in cold and wet soil elsewhere, there is considerable loss of oat seed due to rotting by soil fungi. Observations on a field experiment in 1943 suggested that this loss can also be checked to some extent by the standard organo-mercury seed dressings. On an Annandale farm at an altitude of about 500 ft., Star oat sown in parallel strips of dressed and undressed seed across the field showed reductions in braird on two undressed strips of 66 and 72% respectively, as compared with that from the adjacent treated seed. The amount of leaf stripe visible above ground was very small and combined with these figures gave totals of missing and diseased plants of 69·1 and 73·4%, calculated on a basis of the braird from the treated seed. The treated braird is taken as the basis for calculation because it indicates what the seed was capable of producing when preventable diseases were eliminated. Laboratory tests of the same seed showed it to carry infection with *Pyrenophora avenae* to the extent of 17% and with *Fusarium* spp. to 29%, a total of 46% diseased seed. There was thus a mean loss of seedlings from the untreated seed of 71%, of which only 46% can be accounted for by seed-borne disease. It seems likely that the outstanding 25% loss was due to soil fungi against which the seed dressing afforded protection.

The oat smuts, *Ustilago avenae* and *U. kolleri* may be regarded as curiosities in Scotland to-day, though they may still be found in a few backward districts, especially in the north. As recently as 1914, *Ustilago avenae* was said by Boyd to be 'very common' even in the Forth drainage area and a little earlier he reported it equally common in Ayrshire. The disappearance of the smuts had largely taken place before the introduction of seed dressings and is probably to be attributed to the activities of seed merchants, including the introduction of 'regenerated strains' of oat varieties and to increased care in growth and selection of seed crops. The almost universal practice of change of seed has also no doubt helped to eradicate smut since its underlying idea is to obtain seed from a better crop grown in the districts most suitable for seed production. The only instances of an appreciable amount of smut encountered of late years have been on small farms where the same stock of oats had been grown for a long number of years.

While attention has been concentrated on seed-borne diseases because they offer an easy mode of attack on some of the factors impeding cereal production in Scotland there is also a number of other troubles not amenable to this treatment. The most important of these is take-all, due to *Ophiobolus graminis* var. *avenae*. The prevalence of this disease in the wetter districts of the west and north was not fully appreciated until a crop survey was made in 1942 with Mr Garrett. It was then discovered that whiteheads due to this fungus were often prevalent in oats following old pasture. This was especially the case where the pasture had consisted predominantly of the poorer grasses, mainly *Agrostis* spp. In the south-west and in the hill country of Aberdeenshire and adjacent counties two or even three oat crops are sometimes taken in succession after old grass. In the second, and especially in the third such crop, there are commonly enough whiteheads to cause appreciable loss. In the spring of 1943 much land in Ayrshire was flooded, with consequent loss in fertility by leaching, and crops on such fields proved particularly susceptible to whiteheads, due no doubt to their low nitrogen status.

In the moist climate of the south-west *Ophiobolus graminis* var. *avenae* fructifies readily in the field and by the time the whitened ears become conspicuous the black perithecia with mature ascospores can usually be found in abundance on the base of the straw. Diagnosis of the trouble in the field is thus unusually easy. Severely affected oat plants are often only half as tall as healthy ones and are then liable to be overlooked unless searched for. Hence the incidence of take-all was long under-estimated. These stunted plants usually bear completely empty ears. On the other hand the prematurely bleached ears of slightly affected plants which have attained the same height as the rest of the crop often contain a fair amount of grain. The disease thus appears to be somewhat less destructive in oats than in wheat. On this account, even after old lea, whiteheads seldom cause significant loss in the first oat crop and in the majority of instances noted during the surveys the amount of disease in the second or 'yaval' crop was not serious. The third successive oat crop on land of this type, however, was nearly always severely affected by whiteheads and it seems undesirable to risk more than two successive oat crops except on rich land where the second crop has been found to be virtually free from the disease. Taking a second crop is, however, in some ways desirable, as it utilizes the nutrients released by the rotted turf. As with the wheat strain of this fungus, interposition of a green crop or a potato crop seems to be generally successful in eliminating *Ophiobolus* infection, provided the ground is kept reasonably free from grass weeds.

Stem rust (*Puccinia graminis*) is not uncommon on oat crops in the Tweed valley, where barberry is a common hedge plant. In the rest of the country its occurrence is sporadic and of no consequence. With regard to crown rust (*Puccinia coronata*), the position is rather more interesting. As a rule Scottish oat crops may be said to be completely free from this disease in most districts. At long intervals, however, extensive outbreaks do occur. The most recent one

of any extent was in July 1933. As *Rhamnus* bushes, the alternate hosts, are not native to Scotland and the uredospores apparently do not survive the winter, it seems evident that crown rust outbreaks on our oat crops must originate from wind-blown uredospores from a region where *Rhamnus* is prevalent. Presumably they come from Ireland or the south of England. In the summer of 1943 I found crown rust widespread, though nowhere very severe, on oats in Dumfriesshire, Kirkcudbrightshire, and Wigtownshire. North of this it was found only in a few crops near Dalry, Ayrshire. The restricted spread of this wind-borne disease affords an interesting problem.

Scotland differs widely from England in the relative proportions and distribution of hills and arable land. Instead of being an arable country diversified by hills Scotland is a vast mass of moor and bog relieved by more or less isolated tracts of cultivable ground. Excluding freshwater lochs, the total area of Scotland is 19,069,000 acres, of which arable land in 1937 amounted to only 2,992,028 acres, or only about 15% of the land surface. Thus, an oat disease established, like the crown rust of 1943, in the arable fringe of the Solway Firth has to cross a broad uncultivated tract of more or less high land to reach Ayrshire in the first place. Ayrshire is similarly cut off by a belt of high moor from the Clyde valley and Clydesdale is largely, though not entirely, separated from the Lothians by a desolate wedge of moor and bog. Similarly the Tweed basin is almost completely shut off from the rest of the country by the barrier of the Lammermuirs and adjacent hills. It follows that a wind-borne plant disease, instead of spreading steadily across the country from field to field, has to build up in each area a sufficient body of air-borne inoculum to be carried across a belt of higher ground from 5 to 20 miles wide or more, absolutely free from any host plant. It is to the absence of the alternate host and the obstacles to spread imposed by these natural barriers that I am inclined to attribute the relative freedom of our oats from crown rust. It would be interesting, however, to have detailed information regarding the distribution of the disease in England and especially the date of its first appearance in different counties from south to north. Probably the suggestion that such comparatively paltry land barriers can affect the spread of a rust will not commend itself at once to most people, especially to those familiar with American references to air-borne clouds of uredospores. It has to be remembered, however, that our prevailing winds are westerly and south-westerly, so that the main spore drift from the south of England would not normally pass over Scotland.

As regards wheat diseases there is little to be said. Bunt is hardly ever seen in Scotland, even when infected seed is sown without preliminary dressing with a seed disinfectant. As for loose smut, though affected heads can be found in almost any wheat crop, we have no records of a significant degree of infection. One interesting feature of the wheat rusts is that the common English brown rust, *Puccinia triticina*, is completely unknown in Scotland. Presumably its absence must be due to some inhibitory climatic factor. One would expect an obligate parasite to be adapted particularly to the conditions prevailing over the main areas of distribution of its host and in this connexion it is interesting that Percival quotes 51–52° N. as the limit of profitable wheat culture in Great Britain. The only Scottish record of *P. triticina* was at Coldingham, in latitude 55° 54′ N. Wheat is a fairly common crop to-day on low land all up the east coast, through Berwickshire, the Lothians, Fife, east Perth and Angus and is grown rather extensively still further north in Morayshire. A few enthusiasts are even growing spring wheat in Caithness and Orkney. This is a comparatively modern development but if, as some farmers say, the crop has come to stay in Morayshire, we shall perhaps witness the development of a new race of *P. triticina* adapted to cover this extension in the range of its host.

P. glumarum can usually be found if hunted for and in 1943 it was, as in England, somewhat alarmingly conspicuous. On the whole the outbreak seems to have caused less damage than was expected but in many instances a reduction in yield of 8 bush./acre was estimated. The greatest damage reported is from the Tweed valley, where a crop of wheat which ordinarily would have been estimated at 32 bush./acre actually threshed only 6 bush./acre. Another crop was so badly affected that permission to burn it was sought from the Agricultural Executive Committee, as it would not pay for the cost of harvesting and threshing.

Cercosporella herpotrichoides is now known to be widespread in the Lothians, Fife and Morayshire, where it seems to be responsible for more whiteheads in wheat than is *Ophiobolus graminis*. Extensive lodging of fungus origin is seldom observed in Scotland, probably on account of the long rotation usually practised, but, of late years, with close cereal cropping, instances of 50 and 35% loss of crop have been reported. True take-all, due to *O. graminis*, though widespread along the east coast, is even less important. It is interesting, however, that last year we were able to demonstrate the occurrence of the so-called oat strain of this fungus attacking wheat crops in Dumfriesshire, Kirkcudbrightshire, Wigtonshire, Ayrshire, Fife and Morayshire. Most of these crops showed only 1% or less of whiteheads but in the Morayshire instance a second crop of Wilma wheat on light land showed 8% of whiteheads in the best parts and over large patches of the field half the ears were empty. In most of these districts wheat is a wartime crop only and with many of the infected fields oats had been the previous crop. This may partly account for the absence of the true wheat

strain of *O. graminis* and the presence of its less specialized variety *avenae*.

Scottish barley is grown mainly in the north and its diseases have been little investigated. We do know, however, that both covered and loose smuts are present to such an extent as to cause alarm even to the farmers and seed merchants. Unfortunately the farmers do not differentiate between the two smuts and because loose smut is not controlled by seed dressing they do not think it worth while to dress to eliminate the covered smut. Of fifteen barley seed samples received from the north last year and sown at the Seed Testing Station, Corstorphine, only one was free from loose smut. The others showed from 0·7 to 13% infection with *Ustilago nuda*. Representative heads from each plot were tested by Tapke's spore germination method to check on the possible occurrence of *U. nigra* but none was found. Curiously enough, leaf stripe appears to be not very prevalent in Scottish barleys. Eleven of the fifteen samples were free from it and only one showed as much as 8% infection. If we could eliminate the loose smut the barley situation would be fairly satisfactory. Unfortunately, the practical difficulties in application of the hot water treatment for loose smut have prevented its adoption to clean up the local seed stocks and as there is a strong prejudice against introducing seed from other districts into the north the barley situation has reached an impasse.

Cereal diseases in Mid-Wales

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Mid-Wales, before the war, was chiefly a pastoral province. The acreage under cereal crops fell substantially between 1918 and the present war, wheat disappeared entirely in some districts but oats and barley still remain the principal cereal crops in the province. In 1939 and subsequent years, the arable acreages of the country were increased considerably, and a high percentage of the ploughable acreages of farms is now under rotational crops. This rapid increase in arable acreages has affected the usual rotation of crops and a high proportion of the ploughed land has to bear a succession of straw crops. This intensive cropping with cereals in an area of high rainfall has given rise to a number of interesting plant pathological problems. Some parasitic fungi, which previously never reached epidemic proportions, have to-day become troublesome parasites and cause considerable losses to our cereal crops. For convenience diseases of cereals in Mid-Wales, may be grouped into: (1) rusts and mildew, (2) smuts and bunt, (3) other types.

Rusts and Mildew

Many species of rust fungi have been recorded on cereals, namely, crown rust, *Puccinia coronata* on oats and grasses, yellow rust, *P. glumarum* on wheat and barley, black rust, *P. graminis* on wheat, oats, barley, and species of grasses, and the brown rusts, *P. triticina* on wheat, and *P. anomala* on barley. Of these rust diseases, crown rust is the most important and destructive, and appears every year on oats, rye-grass and other species of grasses. Its incidence on these crops is largely determined by climatic conditions but, with oats, time of sowing and manuring are important factors which determine severity of attack. Late maturing crops usually sustain the heaviest attacks and crops well supplied with nitrogenous fertilizers are more severely affected than crops growing under more natural conditions. Epidemics of crown rust occur at periodic intervals and severe outbreaks occur regularly once every 9 or 10 years, when yield of susceptible varieties may be as low as 5 cwt./acre. All commercial varieties of oats are affected, but the Scotch Potato oat and its related varieties, Castleton and Pure Line potato, are extremely susceptible, and with these varieties, farmers always complain of poor yields in years of epidemics.

Inoculation experiments carried out at the Welsh Plant Breeding Station showed that all commercial varieties (which are included in the *Avena sativa* group) are susceptible to this rust, but many strains and varieties included in the *A. sterilis* group showed a high degree of resistance. Unfortunately, varieties of oats classified in the *sterilis* group possess most undesirable characters such as early ripening, poor tillering capacity, poor grain character, and premature shedding of grain. Investigations are in progress by Mr E. T. Jones, Welsh Plant Breeding Station, to breed a resistant strain of oats. Suitable crosses have been made and promising selections isolated which show a high degree of resistance. The problem, however, is not an easy one, as it is difficult to combine the good qualities of the susceptible parent with the resistance of the *sterilis* parent in the progeny of a selected cross.

The overwintering of crown rust raises an important problem. The alternate hosts, *Rhamnus frangulata* and *R. cathartica*, are conspicuous by their absence in the Mid-Wales Province. Frequent attacks and epidemics cannot, therefore, be attributed entirely to the influence of the alternate host; other means of propagation must exist, and overwintering on volunteer plants cannot be overlooked.

In a moist climate, such as we experience in Mid-Wales, oat grains shed readily at harvest time, germinate quickly, and have every chance of becoming infected by uredospores from other volunteer plants growing around infected crops. Infected plants, therefore, act as centres of infections throughout the winter. From field observations and tests carried out experimentally crown rust fungus can tolerate reasonably low temperatures. Uredopustules, under normal greenhouse conditions, appear in 10 days but may take as long as 24 days to develop under lower temperatures, thus showing that crown rust can persist and tolerate reasonably low temperatures.

The next most important disease is yellow rust, *Puccinia glumarum*, which, with mildew, *Erysiphe graminis*, causes considerable damage to wheat and barley crops. Severe attacks of both fungi are recorded periodically and their combined effect is to reduce the vigour of the crop and, incidentally, yield and quality of grain. Unfortunately, some of our best commercial varieties of wheat are susceptible to both diseases. Wilma and Desprez 80 appear to be the most susceptible varieties, and Jubilee Gem, Yeoman, Juliana, Holdfast, Iron III and Standard Red are moderately susceptible, while Bersée wheat is less susceptible than the other varieties. Mildew appears early in the season on all cereals, and from data collected, stiff straw varieties are most severely affected.

Black or stem rust, *Puccinia graminis*, is another type which is often seen on cereals and grasses. Its alternate host, the common barberry, is well distributed in the province. Specialization of the rust may be seen in the field when cereal crops may be perfectly free from any traces of the disease, but bent and other grasses, growing as weeds, may be severely affected. Again, the barberry may be covered with aecidia cups, but no signs of the rust may be seen on its usual hosts, thus showing that infection of crops is largely determined by climatic conditions at the time of aecidiospore dispersal. The brown rusts, *P. triticina* and *P. anomala*, are observed every year but are of no economic importance.

Bunt and Smuts

All the common forms are present on the three cereal crops, *Tilleta caries* on wheat, *Ustilago avenae* and *U. kolleri* on oats, and *U. hordei* on barley, are the most important types. Occasionally, a crop of wheat is so severely affected by bunt that the whole of the grain is rejected for use as human food. Some of these attacks are attributed to threshing machines which operate from farm to farm. In an area of comparatively small producers there is often a danger of threshing machines carrying bunt spores from one farm to another, thus bringing about contamination of crops. Fortunately, the increasing use of modern seed dressings is an excellent safeguard against transmission of bunt and similar seed-borne diseases. Both smut diseases of oats are prevalent in the area. *U. avenae* is usually found on *sativa* varieties, and *U. kolleri* on *strigosa* varieties, and severe attacks of the latter are recorded on *strigosa* oats. This is attributed to the difficulty of dressing these varieties with the usual dust treatments. Grain of *strigosa* varieties is covered with fine hairs and twisted awns, and does not readily pass through a large modern seed-dressing machine, consequently, there is less seed dressing done with *strigosa* than *sativa* varieties.

Other Types

In the third group of parasitic fungi, take-all *Ophiobolus graminis*, and its related variety, *avenae*, are now well established on cereal crops in the Province. Both types are widely distributed, which is attributed to high rainfall and the frequency with which cereals are now grown in a rotation. Field observations show that the perfect stage of the take-all fungus develops around the base of the stem and in the cortical tissues of the crown roots at the end of July. Perithecia are fully developed at the end of September, when ascospore discharge usually begins and continues until December. At this time ascospores are difficult to find in the field. Discharge, therefore, coincides with the sowing period of a winter cereal crop, and ascospores, on germination, are probably responsible for seedling infection at this time of the year. On the other hand, infection of spring-sown cereals is probably the result of contact between young seedlings and infected roots and stolons of carrier hosts. Already take-all has been found on the root systems of the following plants: crowfoot, milfoil, marsh foxtail, and white clover. Weeds, therefore, act as important agents in perpetuating the take-all fungus from year to year. A survey of thirty farms, made in the province last year where wheat crops had failed, revealed many interesting points which are summarized as follows:

(1) *Ophiobolus* was more prevalent in crops growing after grass than in crops growing after stubble. This conclusion may not agree with the findings of Buddin & Garett, but in view of the importance of weeds as carriers of this fungus, it is possible that pastures in a humid climate may be more heavily infected with carrier weeds than pastures situated in areas of lower rainfall. Some of the most severely affected crops of oats last year were recorded in fields which had not been ploughed since the last war.

(2) *Ophiobolus* was associated with all wheat failures, but results show that other factors may be responsible for loss of crops.

(3) Only the form *avenae* was present on crops examined.

(4) Wheat and oats can tolerate *Ophiobolus* infection and grow vigorously. Typical mycelial growth

of the fungus was often seen on the roots of matured plants without any sign of basal infection of tillers.

(5) Lime encouraged the incidence of *Ophiobolus*.

Leaf spot caused by *Helminthosporium avenae* is recorded regularly on oat crops in the province. It does considerable damage in the seedling stage and many crop failures are attributed to the severity of infection. All varieties of oats are attacked, but heavy yielding types are particularly susceptible and, with these varieties, organo-mercury seed dressings give outstanding results. Treated seed gives better establishment, uniform and more vigorous growth.

Another disease recorded for the first time in Mid-Wales is ear blight, *Giberella zeae*, which was frequently found on wheat, oats and barley during the exceptionally wet seasons of 1942 and 1943. The fungus was often seen on heads which had become damp through heavy rainfall. From general observations recorded during these seasons, the disease appears to be more prevalent on laid and weather beaten crops. Under these conditions it may develop on the straw or ears before cutting, or it may even develop in the stook if the crop is damp and sodden.

In addition to the above-mentioned types, other, but less destructive diseases, are regularly observed on cereal crops, and amongst these, may be mentioned *Septoria tritici* and *nodorum* on wheat, *Fusarum* sp., causing foot-rot troubles in barley and oats, *Helminthosporium gramineum* and *H. teres*, causing leaf-stripe and blotch on barley.

The take-all disease of cereals

By S. D. Garrett, *Rothamsted Experimental Station, Harpenden, Herts.*

Soil and other environmental conditions affect the take-all disease of cereals in three principal ways. First, they determine the rate of subterranean spread of the causal fungus, *Ophiobolus graminis*, along the roots of its host plant, and, secondly, they affect the host plant's reaction to infection; thirdly, they influence the survival of the fungus in infected root and stubble residues left behind in the soil after harvest.

Growth of the runner hyphae of *Ophiobolus* along the host roots is favoured, first, by good soil aeration, and, secondly, by an alkaline reaction of the soil. It is possible that rate of growth of the runner hyphae in poorly aerated or acid soils is controlled by accumulation of respiratory carbon dioxide at the root surface, with which must be associated a reduction in the partial pressure of oxygen. In well-aerated soils, carbon dioxide must diffuse rapidly away from the root zone, and in alkaline soils, a high proportion of the carbon dioxide is transformed into bicarbonate. Good correlation has been obtained between conditions found to favour a rapid growth of runner hyphae along the host roots, and those known to promote development of take-all in the field.

Light-textured soils also favour the development of take-all in a second way, inasmuch as they are usually poor in nitrogen, and often in other plant nutrients as well. A cereal plant growing in a soil well supplied with nutrients can produce many new secondary roots to replace those destroyed by *Ophiobolus*, but a deficiency of one or more of the three major nutrients, nitrogen, phosphorus or potassium, will curtail this power of recovery, and lead to early death of the diseased plant. In Australia, phosphate is most commonly the nutrient in short supply, whereas in England nitrogen is usually the missing nutrient. The exceptionally widespread outbreaks of take-all observed by Mr Buddin and myself in the Southern Advisory Province in 1943 may have been due in part to leaching-out of nitrates by the heavy January rainfall, which was double the average in that year. A coincidence of patches of the take-all disease with low-lying areas liable to be flooded in winter may perhaps be due to such a leaching-out of nitrate nitrogen from the soil.

Survival of *Ophiobolus* in infected root and stubble residues is dependent chiefly upon an adequate supply of nitrogen from the surrounding soil. Under such conditions, the mycelium of *Ophiobolus* is enabled to form new branch hyphae, which can explore non-eroded cell tissue, and so the fungus continues to survive.

Eyespot, *Cercosporella herpotrichoides* Fron, and lodging of wheat

By Mary D. Glynne, *Rothamsted Experimental Station, Harpenden, Herts*

Eyespot of wheat, caused by the fungus *Cercosporella herpotrichoides* Fron, has been well known to farmers for a long time under such dialect names as straggling, scrailing, shakely, brackley and scrawly. The disease is widespread in Europe and in parts of America, is found in Australia and seems to be serious in New Zealand, favoured no doubt by the rather high rainfall, well distributed throughout the year. It is found in England, Wales and Scotland, increasing from west to east as the frequency of wheat and barley growing increases. It is most serious on winter sown wheat and barley; when sown in spring these crops are seldom seriously affected though they help to carry on the disease; oats is highly resistant.

The fungus persists on infected stubble, on which it produces spores freely from autumn to spring. The spores are carried in blown rain and infect young plants on which more spores are produced which cause secondary infection of surrounding plants. The fungus penetrates successive leaf sheaths, eventually reaching the culm. Eyespot, like take-all, produces white or prematurely ripened heads scattered through the crop before harvest.

The ripe straws tend to bend or break in the middle of the eyespot lesion. In a thin crop this results in individual straws falling down in all directions among the upright ones; this is the condition known to farmers as straggling. In a heavier crop, weakening of straws by eyespot may result in general lodging. The tendency to lodge increases both with the weight of crop and with the percentage infection by eyespot. While very heavy crops may lodge when not infected, less heavy ones go down when severely infected by eyespot; so that the disease increases both the probability and the earliness of lodging.

To estimate the loss caused by eyespot, we need to know both the direct loss due to the disease and the indirect loss resulting from lodging, as well as the distribution and percentage of disease in the wheat crops of the country and the percentage area lodged by eyespot each year. At Rothamsted, Wendy Dion, Joan Thurston and I have been fortunate in having the collaboration of a statistician, J. W. Weil, in experiments which are giving information on the loss resulting from eyespot and from lodging. A limited survey of wheat crops in 1941 showed that eyespot is common in the south-eastern counties of England; far more extensive surveys are needed to determine the effect of the disease in the country as a whole.

Pot experiments in which infected plants were compared with healthy ones, showed that whereas nearly all the latter survived; the loss of plants through death amounted to 23% at a low level of soil fertility and when nitrogen was supplied, this was reduced to 11%. The reason for the difference lies in the greater production of tillers in the well-nourished plants which enables them to throw off the more badly diseased and retain the cleaner tillers. The loss in total yield of grain was 44% in the low and 16% in the high nitrogen series. Thus, although nitrogenous manures may increase the amount of eyespot evident at harvest, they help the plant to resist the effects of the disease and to give increased yields.

In 1943 an experiment was carried out in which eyespot in a heavily infected field was reduced by 10% by spraying in the autumn, with sulphuric acid. Associated with the decrease in eyespot there was an increase in yield of 18%, equivalent to about 3 cwt. of grain per acre. This effect, though no doubt partly due to reduction of disease, may also have been partly due to other favourable effects of the treatment, such as reduction of weeds. In small-scale field experiments by Wendy Dion in which weeds were removed, spraying with sulphuric acid in spring resulted in an increase of 33% total grain associated with a decrease of 31% straws severely infected by eyespot. The loss indicated by these figures amounts to more than 1% of grain for 1% straws severely infected by eyespot at harvest, which cannot be true over the whole range, as 100% infection does not result in complete loss. It may be true for part of the range of infection, if the relation is represented by a sigmoid curve, in which the greatest loss is in the middle part of the curve. Alternatively, the treatments for reducing eyespot may have other favourable effects on the crop.

An experiment now in progress eliminates the effects of treatment apart from disease; a clean field has two blocks untreated and two infected with eyespot from stubble and infected plants put on the plots in autumn. This should give an estimate of the effect of moderate infection on four varieties sown at two dates in 1944 and will be followed by an experiment in which higher rates of infection are attempted.

In the course of one experiment some 20,000 straws were obtained at harvest, representing random samples from 128 plots, amounting to the produce of about $\frac{1}{60}$ acre. Healthy straws and those with superficial lesions yielded twice as much grain per straw as did severely diseased ones. On this basis the loss in yield would be 0·5% grain for every 1% straws severely infected at harvest; so that a field with 60% badly infected straws would have lost about 30% of its grain. There is a good deal of general evidence from farmers' views and from experimental work

that this is a reasonable figure. Further loss is entailed because the size of grain produced by diseased plants is less and so there is more tail corn from diseased than from healthy plants.

Loss from lodging, independent of eyespot, has been determined by inducing lodging at different dates before harvest in replicated plots. The earliest lodging resulted in the greatest loss, amounting to 54% of the grain in plots lodged 6½ weeks before harvest and to 29% four weeks later. In this experiment there was no bird damage, but in a subsequent trial, birds increased the loss from lodging to 74%. Uninfected crops which lodge tend to fall in one direction and so can be harvested by working one way with the binder, but when they are lodged as a result of eyespot infection they fall in all directions and have to be harvested by scythe. Eyespot, by increasing the tendency to lodge and by making it occur earlier in the season, may thus cause great loss in yield and a large increase in cost of harvesting.

The wheat yield of this country could be greatly increased by the more generous use of fertilizers, but farmers are afraid of lodging. This danger can be avoided by using short or stiff strawed varieties, whose chief advantage is, however, lost unless they are manured up to a level of soil fertility at which long strawed kinds are likely to lodge. The best means of controlling eyespot is by sound rotations; ley farming in which arable and grass alternate every few years seems admirable for the control of both eyespot and take-all. Spraying with sulphuric acid for the reduction of eyespot in crops already infected, is still in the experimental stage and cannot yet be recommended for general use. It has given promising results especially when applied in early spring, but when put on as late as April and May sometimes reduces yields. Further trials in different centres and at different times of year are needed.

Two other diseases resemble eyespot, though their effects are less serious; Sharp eyespot, *Corticium* (*Rhizoctonia*) *solani*, which is common but seldom severe, and white straw disease, *Gibellina cerealis*, hitherto found in this country only on one field at Rothamsted.

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1929 Findlay, W. P. K., D.Sc., A.R.C.S., D.I.C., Forest Products Research Laboratory, Princes Risborough, Bucks. (Botanical Secretary 1936; General and Botanical Secretary 1937– .)
1943 Finney, D. J., M.A., Rothamsted Experimental Station, Harpenden, Herts.
1935 Fish, S., M.Agr.Sc., Department of Agriculture, Biological Branch, Plant Research Laboratory, Burnley Gardens, Swan Street, Burnley, E. 1, Victoria, Australia.
1919 Fisher, K., The School, Oundle, Northampton.
1923 Fisher, R. C., B.Sc., Ph.D., Forest Products Research Laboratory, Princes Risborough, Bucks. (Vice-President 1936; Council 1934–6.)
1942 Fitzgibbon, M., A.I.C., F.C.S., Lunevale Products, Ltd., Queens Mill, Lancaster.
1931 Foister, C. E., B.A., Ph.D., Seed Testing and Plant Registration Station, East Craigs, Corstorphine, Edinburgh, 12.
1920 Fox-Wilson, G., N.D.H., F.L.S., R.H.S. Laboratories, Wisley, Ripley, Woking, Surrey. (General and Zoological Secretary 1935–6; Zoological Secretary 1933–4, 1937–8; Council 1927–9.)

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1913 Fryer, J. C. F., O.B.E., M.A., Agricultural Research Council, 6*a* Deans Yard, Westminster, S.W. 1. (President 1926–7; Treasurer 1914–19; Council 1921, 1924–5.)
1933 Gadd, C. H., D.Sc., Tea Research Institute of Ceylon, St Coombs, Talawakelle, Ceylon.
1930 Galloway, L. D., M.A., c/o Midland Bank, Wimbledon Hill, London, S.W. 19.
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1944 Gemmell, A. R., B.Sc., M.S., Ph.D., Botany Department, The University, Glasgow, W. 2.
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1937 Gillespy, T. G., B.Sc., A.R.C.S., University of Bristol Fruit and Vegetable Preservation Research Station, Campden, Glos.
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1941 Glasscock, H. H., M.Sc., Dip. Agric., South-Eastern Agricultural College, Wye, Kent.
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1920 Goodey, T., D.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans, Herts. (President 1934–5; Council 1923–9, 1936–8.)
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1920 Gwynne-Vaughan, Prof. Dame Helen, G.B.E., D.Sc., LL.D., F.L.S., Birkbeck College, Chancery Lane, London, E.C. 4.
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1942 Hewlett, P. S., B.Sc., Pest Infestation Laboratory, Biological Field Station, Slough, Bucks.

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1935 Hodson, W. E. H., A.R.C.S., D.I.C., Agricultural Advisory Offices, The University, Reading. (Council 1941–2.)
1937 Holborn, J. M., B.Sc., A.R.C.S., Jeyes' Sanitary Compounds Co. Ltd., Richmond Street, Plaistow, London, E. 13 (temporarily at 129 Trinity Road, London, S.W. 17).
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1927 Jacobs, S. E., Ph.D., Bacteriology Department, Imperial College of Science, South Kensington, London, S.W. 7. (Council 1942–3.)
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1940 Keay, Miss M. A., M.A., Ph.D., H.M. Flax Establishment, Flitcham Abbey, nr. King's Lynn, Norfolk.
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1934 Lean, O. B., B.Sc., D.I.C., Imperial Chemical Industries, Ltd., Jealott's Hill Research Station, Bracknell, Berks. (Council 1940–2.)
1926 Le Pelley, R. H., B.Sc., Ph.D., D.I.C., A.R.C.S., Department of Agriculture, Nairobi, Kenya, E. Africa.
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1936 Macdonald, J. A., B.Sc., Ph.D., F.R.S.E., Botany Department, The University, St Andrews.

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1920 MANGHAM, Prof. S., M.A., University College, Southampton.
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1940 MARTIN, J. T., D.Sc., F.I.C., Plant Pathology Laboratory, Milton Road, Harpenden, Herts.
1922 MASON, E. W., M.A., M.Sc., F.L.S., Imperial Mycological Institute, Kew, Surrey. (Vice-President 1943–4; Council 1943– .)
1927 MASSEE, A. M., D.Sc., East Malling Research Station, nr. Maidstone, Kent. (Council 1932–4.)
1938 MEGGITT, F. J., M.Sc., Ph.D., Department of Zoology and Botany, University College, Rangoon.
1933 MEIKLEJOHN, Miss J., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
1943 MELLOR, H. C., B.Sc., A.R.C.S., Bayer Products, Ltd., Agricultural Laboratory, 272 Colney Hatch Lane, Friern Barnet, London, N. 11.
1941 METCALFE, C. R., M.A., Ph.D., Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey.
1923 MILES, H. W., D.Sc., Ph.D., Research Station, Long Ashton, Bristol. (Vice-President 1939, 1943–4; Council 1937–9, 1943– .)
1921 MILLARD, W. A., D.Sc., Department of Agriculture, The University, Leeds, 2.
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1940 MONTGOMERY, H. B. S., B.A., Ph.D., D.I.C., East Malling Research Station, nr. Maidstone, Kent.
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1940 MOORE, M. H., Dip. Hort., East Malling Research Station, nr. Maidstone, Kent.
1928 MOORE, W. C., M.A., Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (Treasurer 1940 ; Council 1938–9.)
1941 MORISON, G. D., B.Sc., Ph.D., Advisory Entomological Department, Marischal College, Aberdeen.
1922 MORLAND, D. M. T., M.A., Seale-Hayne Agricultural College, Newton Abbot, Devon.
1944 MORLEY, B. D. W., B.A., School of Agriculture, Cambridge.
1920 MORRIS, H. M., M.Sc., Agricultural Department, Nicosia, Cyprus.
1920 MOSLEY, F. O., F.L.S., Research Laboratory, The Nurseries, Uxbridge, Middx.
1914 MUNRO, Prof. J. W., M.A., D.Sc., Zoology Department, Imperial College of Science, South Kensington, London, S.W. 7. (Vice-President 1933; General and Zoological Secretary 1928–32; Zoological Editor 1933; Council 1922–4, 1927.)
1938 MUSGRAVE, A. J., B.Sc., D.I.C., Imperial College Biological Field Station, Slough, Bucks.
1925 MUSKETT, Prof. A. E., D.Sc., A.R.C.S., Plant Disease Division, Queen's University, Elmwood Avenue, Belfast, N. Ireland.
1937 NATTRASS, R. M., B.Sc., Ph.D., D.I.C., Department of Agriculture, Nairobi, Kenya, E. Africa.
1928 NEWTON, H. C. F., A.R.C.S., D.I.C., Harper Adams Agricultural College, Newport, Salop. (Council 1938–40.)
1943 NEWTON, LILY, M.Sc., Ph.D., Botanical Department, University College of Wales, Aberystwyth.
1944 OGG, W. G., M.A., B.Sc., Ph.D., F.R.S.E., Director, Rothamsted Experimental Station, Harpenden, Herts.
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1925 OLDHAM, J. N., B.Sc., Ph.D., Research Institute in Animal Pathology, Royal Veterinary College, The Beeches, Streatley, Berks. (Council 1934.)
1940 ORDISH, F. G., Dip. Hort., Plant Protection, Ltd., Yalding, Kent.
1943 OXLEY, T. A., B.Sc., A.R.C.S., Pest Infestation Laboratory, Biological Field Station, Slough, Bucks.
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1935 PEARL, R. T., B.Sc., A.R.C.S., Park Cottage, Hook Green, Tunbridge Wells.

1914 PETHERBRIDGE, F. R., M.A., School of Agriculture, Cambridge. (Vice-President 1940–1. Council 1939–41.)
Orig. PETHYBRIDGE, G. H., M.A., B.Sc., Ph.D., Penlee, Harleigh Road, Bodmin, Cornwall. (Vice-President 1926–7; Council 1912–13, 1925–7.)
1943 PHILLIPS, Miss R. M., Dip. Hort., Churchfields, Stonesfield, Oxford.
1942 PLANT, W., B.Sc., Ph.D., N.D.H., Research Station, Long Ashton, Bristol.
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1942 PRENTICE, I. W., B.Sc., East Malling Research Station, nr. Maidstone, Kent.
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1918 ROBSON, R., M.Sc., Essex Institute of Agriculture, Writtle, Chelmsford.
1920 ROEBUCK, A., N.D.A., Midland Agricultural College, Sutton Bonington, Loughborough, Leics. (Council 1938–40.)
1944 ROGERS, W. S., M.A., Ph.D., East Malling Research Station, nr. Maidstone, Kent.
1932 RUSSELL, T. A., B.Sc., A.R.C.S., Department of Agriculture, Paget East, Bermuda.
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1933 SCHIMMER, Miss F. C., M.Sc., A.R.C.S., D.I.C., Swanley Horticultural College, Ryde House, Ripley, Surrey.
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1938 SHAW, H., M.Sc., Ph.D., East Malling Research Station, nr. Maidstone, Kent. (Council 1943.)
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1932 SHEFFIELD, Miss F. M. L., D.Sc., Ph.D., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.
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1920 SMITH, K. M., D.Sc., Ph.D., F.R.S., Director, Plant Virus Research Station, School of Agriculture, Cambridge.
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1920 SPINKS, G. T., M.A., Research Station, Long Ashton, Bristol.
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1933 STEER, W., M.A., Entomology Laboratory, The University, Manchester, 13. (Council, 1942–4.)
1922 STIRRUP, H. H., M.Sc., Midland Agricultural College, Sutton Bonington, Loughborough, Leics.
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1914 TAYLOR, H. V., O.B.E., V.M.H., D.Sc., Commissioner for Horticulture, Ministry of Agriculture and Fisheries, Africa House, Kingsway, London, W.C. 2. (Council 1927–8.)
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1927 THAYSEN, A. C., Ph.D., Microbiological Section, Chemical Research Laboratory, Teddington. (Council, 1933–5.)
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1938 TURNER, Miss E. M. C., B.A., Water Pollution Research Laboratory, Langley Road, Watford, Herts.
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